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BULLETIN

OF THE NEW YORK ACADEMY OF MEDICINE

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A Symposium

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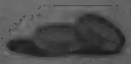

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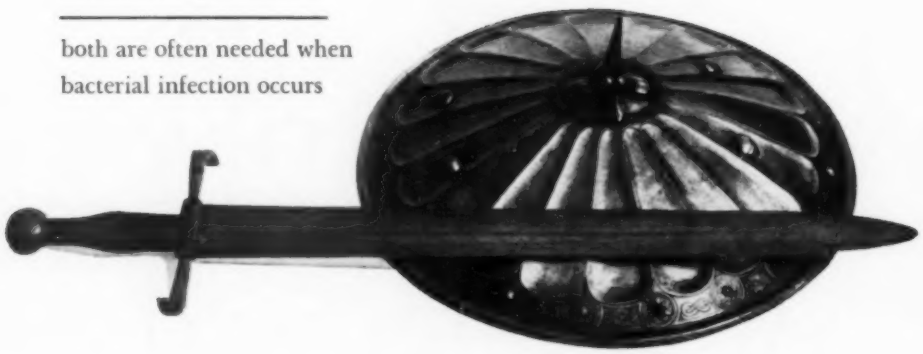

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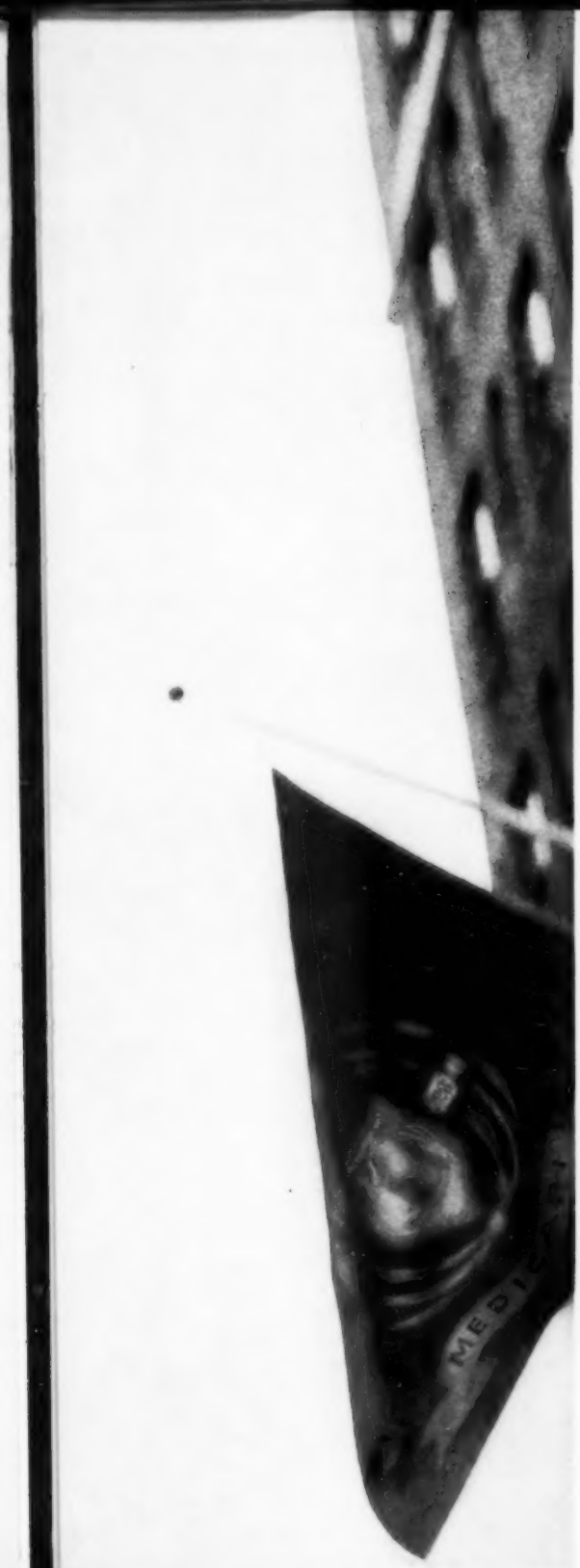

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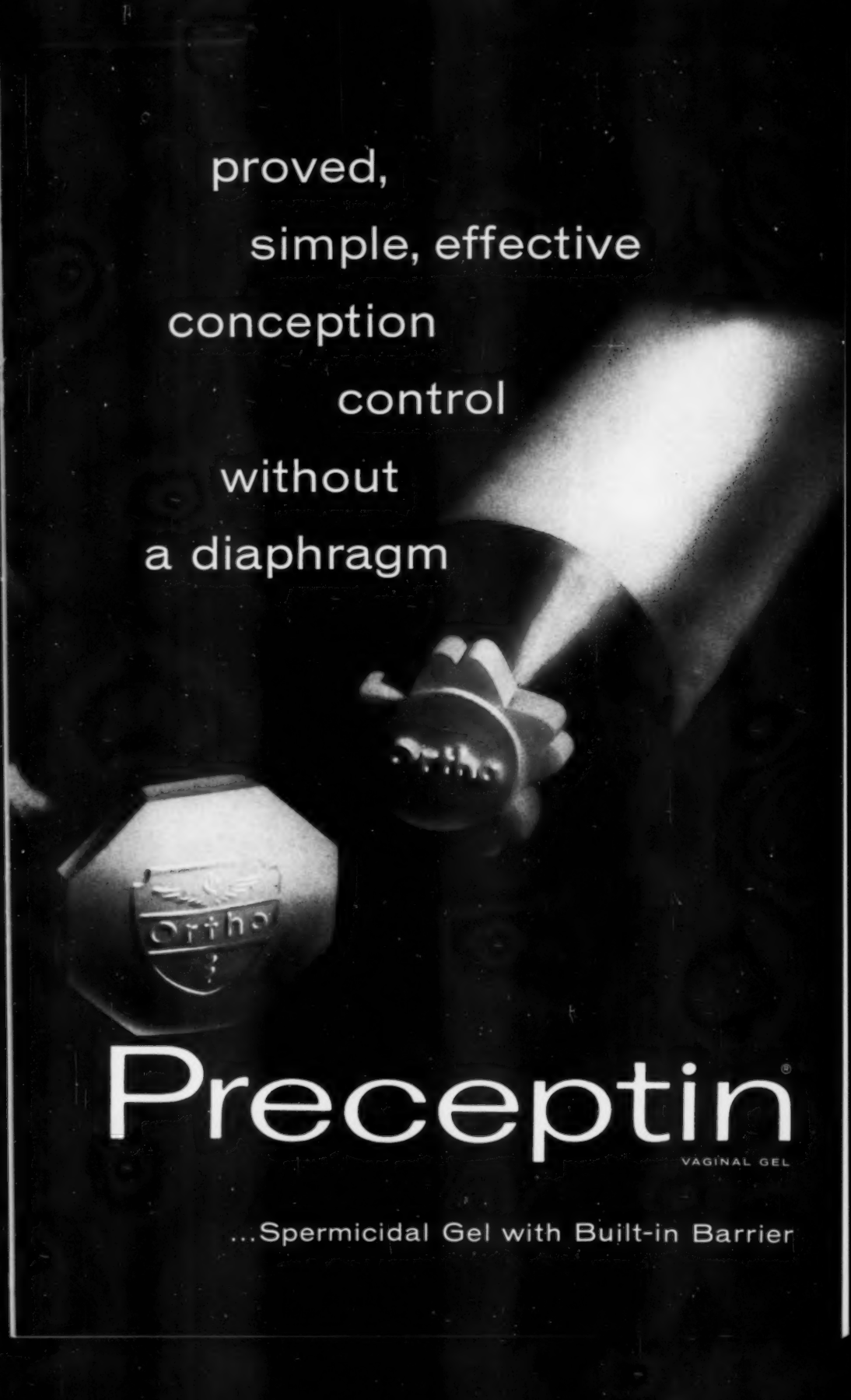
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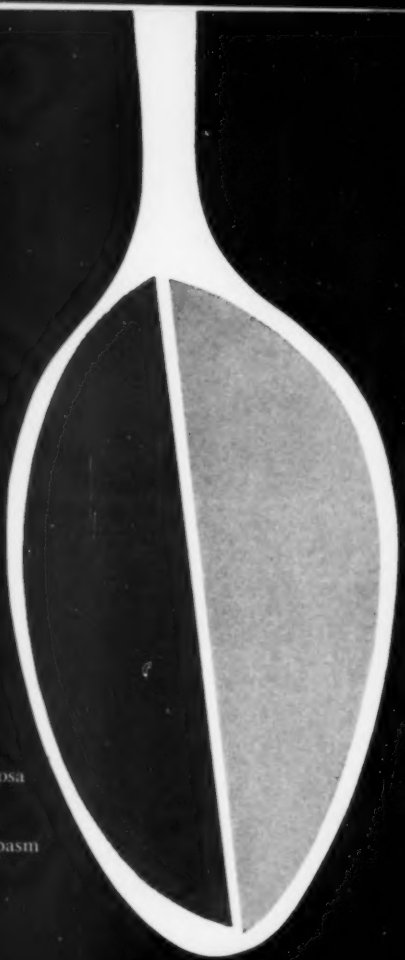

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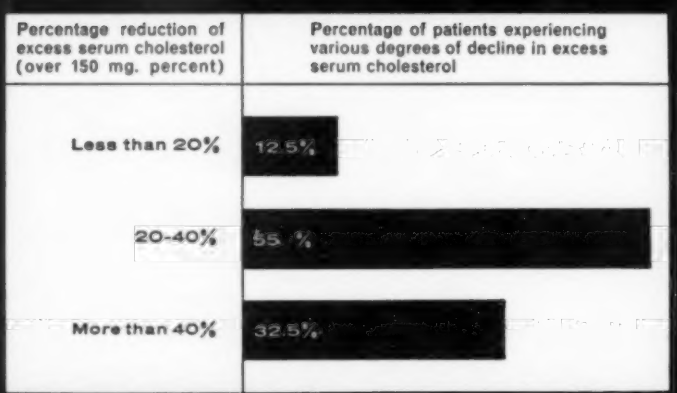
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AUTHORS ALONE ARE RESPONSIBLE FOR OPINIONS EXPRESSED IN THEIR CONTRIBUTIONS

ROBERT L. CRAIG, *Editor*

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BULLETIN OF
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VOL. 34, NO. 11 NOVEMBER 1958

THE CONNECTIVE TISSUES IN MEDICINE AND SURGERY*

JEROME GROSS

Assistant Professor of Medicine, Harvard Medical School;
Associate Biologist, Massachusetts General Hospital, Boston, Massachusetts;
Established Investigator of the American Heart Association

CURRENT research activity in the connective tissue field owes much of its initial vigor to the enticing concept of the "connective tissue" or "collagen" diseases developed by Klinge, Klemperer and others. I wish it were possible to claim that we understand how connective tissue is involved in these conditions or at least to report significant progress in this direction. Witness the gap between infection with the B hemolytic streptococcus and the scarred distorted heart valves in rheumatic fever. In the opinion of this writer there is little evidence as yet that collagen is involved in any way, other than as an innocent bystander or in the healing accompanying injury. The significance of the variable and often very minimal changes observed in the ground substances are yet to be evaluated. Even in scleroderma, a disease manifesting a gross alteration in connective tissue, both qualitative and quantitative informa-

* Presented at the Postgraduate Week, *Research Contributions to Clinical Practice*, of The New York Academy of Medicine, October 9, 1957.

From the Department of Medicine, Harvard Medical School at the Massachusetts General Hospital. This is publication #230 of the Robert W. Lovett Memorial Foundation for the Study of Crippling Disease. Much of the work described herein has been supported by Grant #A90 of the National Institute of Arthritis and Metabolic Diseases and C3638 of the National Cancer Institute of the United States Public Health Service.

tion concerning the changes in the tissue components is still lacking. There is only histological evidence, to date, for an increase in collagen content in the skin. Although fibrinoid has long been considered the hallmark of connective tissue diseases there is still no general agreement as to either its composition or its pathogenetic significance[1-4]. Is it an important part of the disease process with regard to manifestations? Is it a good lead to the mechanism? Or does it represent burned-out ashes of a relatively non-specific process?

Perhaps one measure of the current state of our knowledge concerning pathology of the connective tissue is our understanding of the nature of that most legitimate of all connective tissue diseases, scurvy. The cause and cure are known; an ideal experimental animal, the guinea pig, is readily available; the disease may be turned on and off at will and its severity is measurable. Since the original observations of Wolbach in the early 1930's, progress with regard to pathogenesis has been painfully slow. We have more descriptive information of a biochemical nature and several interesting hypotheses but the mechanisms responsible for manifestations of scurvy, such as failure to heal, hemorrhage, loosening of teeth and muscle attachments, etc. are still obscure. The biochemical functions of vitamin C are still unknown. Our difficulties with the pathogenesis of the rheumatic diseases are therefore not surprising.

Whether the connective tissue is a prime reactor in the rheumatic diseases or involved secondarily, the crippling deformities and much of the functional impairment result from failure of the newly forming connective tissue to reproduce the normal structures. Although this healing phase may be non-specific it is none the less a major feature of the course and ultimate consequences of most of this group of diseases.

This paper will deal with two specific problems related to healing, in which the writer has been involved, namely, collagen formation and wound contraction. There are a number of excellent connective tissue reviews now available[5-10].

Fibrogenesis of Collagen

The mechanism of collagen formation has intrigued histologists, pathologists and surgeons for many decades. Much discussion has centered on the question as to whether or not the collagen fibrils are formed intra- or extracellularly. This old argument is still viable and difficult

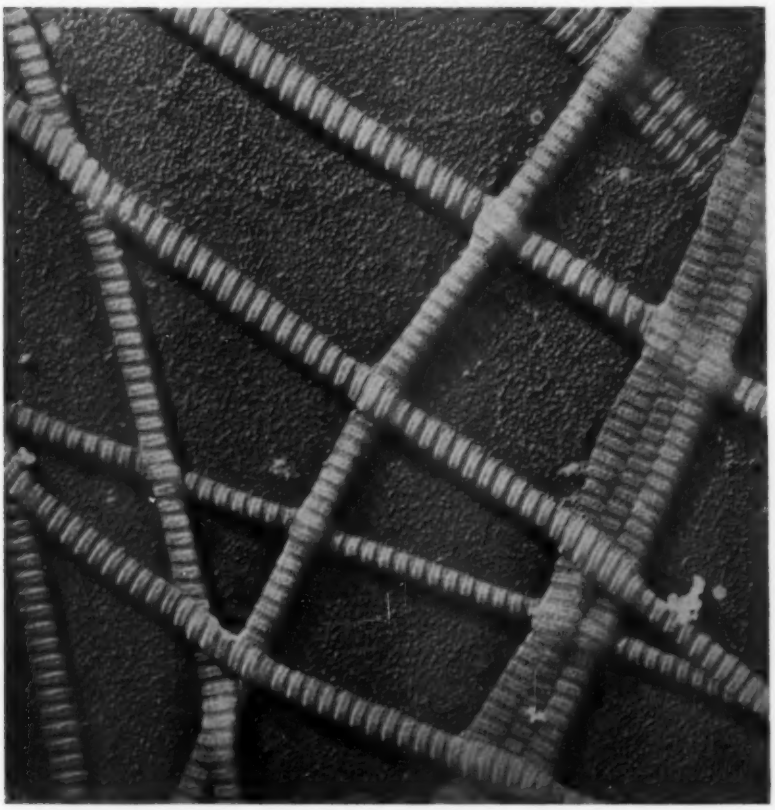

PLATE I—Electron micrograph of collagen fibrils from bovine skin. Shadowed with chromium. 47,000 X.

to settle conclusively—even by electron microscopy. The demonstrations of collagen fibrils within the fibroblasts, clearly observed on occasion in electron micrographs of thin sections[13, 17a], may be interpreted as representing collagen enveloped by the cell in its passage through the tightly packed "jungle" of fibrils. They may also represent precipitation of fibrils within a vacuole of extracellular matrix which had been pinocytosed by the cell. Light optical studies of fibroblasts in tissue culture have suggested fibrous structures coming directly from the cells; these have been widely interpreted as representing collagen fibers formed within the cell and in the process of being extruded[17b]. Electron

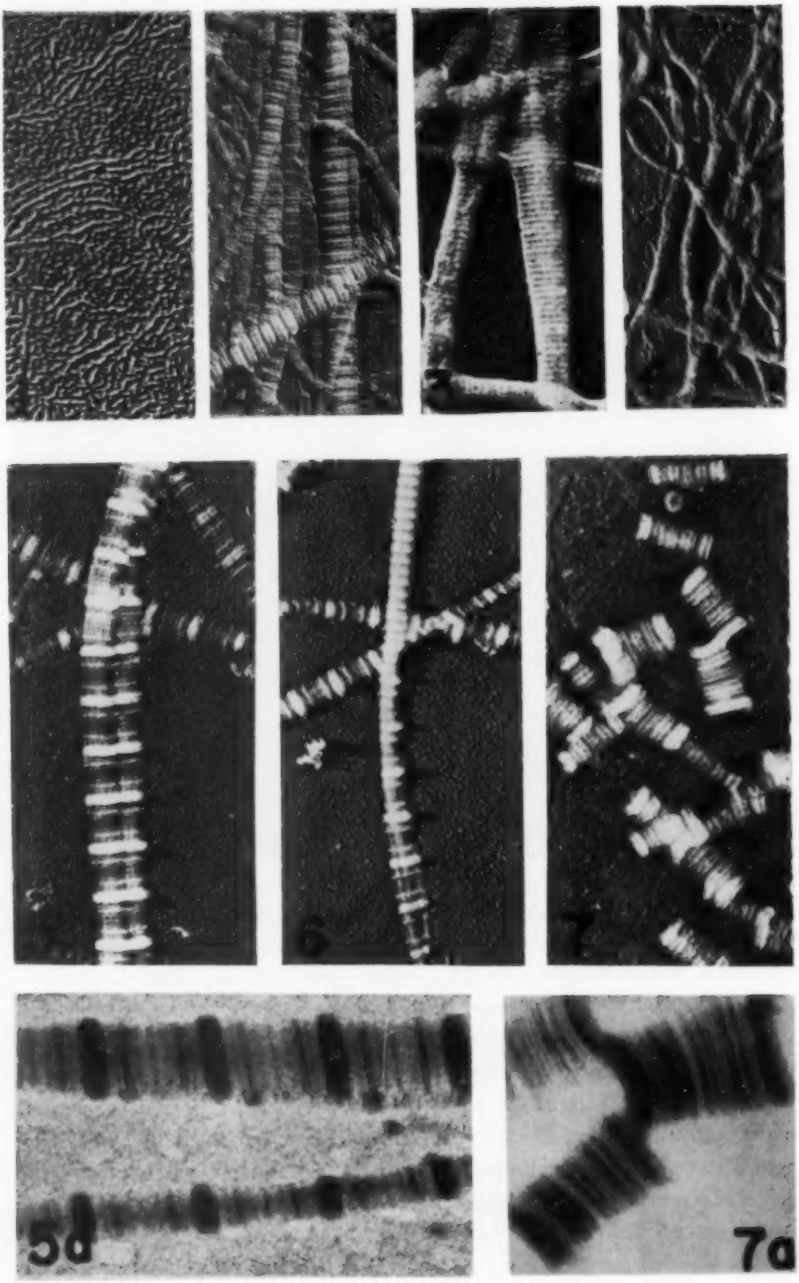

PLATE II [*LEGEND ON OPPOSITE PAGE*]

micrographs have demonstrated that these structures are usually protoplasmic extensions and cannot be identified as collagen (Plate I). The formation of fibrils close to such protoplasmic processes or close to the cell surface could readily cause confusion on the histological level. It would not be too surprising, however, if under certain special circumstances collagen fibrils were formed inside the cell.

The case for extracellular formation rests on direct observations in tissue culture[18] and in the rabbit ear chamber[19] and by inference, from *in vitro* experiments demonstrating reconstitution of fibrils from collagen solutions in the test tube[20-23]. More than 50 years ago it was discovered that a portion of certain tissues such as tendon and skin collagen may be dissolved in dilute acid and reconstituted to fibrils simply by the addition of salt or neutralization to physiologic ionic strength and pH. These fibrils, as seen in the electron microscope, show all the detailed fine structure of the native collagen (Plate II, Figures 1 and 2). It is also possible to reconstitute collagen from solution by various manipulations[24-27] in the form of structures with entirely different morphology (Plate II). Fibrils without period or with axial periodicities of 220 Å or 2500 Å may be precipitated from solution. Sheets of collagen with the native 640 Å period or the artificial 2500 Å may be formed from solution and also small regular crystallities 2500 Å long with an intricate and reproducible fine structure. These different forms

---

PLATE II—(From Gross, J. J. Biophys. Biochem. Cytol., 2:261, 1956. Suppl. Reprinted by permission.)

Fig. 1. Acetic acid solution of collagen, pH 5.0; chromium-shadowed. X 50,000.

Fig. 2. Collagen fibrils precipitated from collagen solution by 1 per cent NaCl. Average period 650 Å. X 32,000.

Fig. 3. Collagen fibrils precipitated from collagen solution by 2 per cent NaCl. X 45,000.

Fig. 4. Collagen fibrils precipitated from collagen solution by 3 per cent NaCl. X 30,000.

Fig. 5. FLS (Fibrous Long Spacing) precipitated from collagen solution by addition of serum α_1 acid glycoprotein, 0.06 per cent, followed by dialysis. (Other inducing agents will produce the same structure; only the collagen is specific.) X 30,250.

Fig. 5a. Same but stained with phosphotungstic acid. Note symmetrical fine structure. X 103,000.

Fig. 6. Intermediate fibril type, native collagen period transforming to FLS. Induced by addition of 0.03 per cent glycoprotein. X 26,000.

Fig. 7. SLS (Segment Long Spacing) precipitated from collagen solution by addition of 0.2 per cent ATP. X 38,500.

Fig. 7a. Same as Fig. 7 but stained with phosphotungstic acid. X 117,000.

of collagen represent different states of aggregation of the highly asymmetric collagen molecule, called tropocollagen[25-26]. The inducing agents are relatively non-specific. This fundamental unit of collagen is a rigid rod about 2800 Å in length and 17 Å wide as measured in solution by physical chemical methods[28] and visualized directly in the electron microscope[29]. The structure of the molecule itself is under intensive investigation primarily by the methods of x-ray diffraction and physical chemistry[16].

These studies indicate that a solution of collagen molecules may be aggregated reversibly, without the intervention of cells or enzymes, into a variety of structural forms including that of the native collagen fibril found in tissues. Indeed, this is the form which almost invariably occurs when the pH and ionic strength are in the physiological range. All the other types require additional conditions. The type of fibril with a 220 Å period has been reported in embryonic tissues and in tissue cultures, the structureless form has been seen in cartilage and nerve, and something resembling the "fibrous long spacing" with periods of the order 1800 Å have been seen in cornea[30]. These unusual forms of collagen may reflect unusual environmental conditions at the time of their formation.

Collagen has generally been considered to be an insoluble substance and has thus been classified among the scleroproteins, however some of its most interesting properties have been discovered in the course of studies on its solubility in aqueous media. The observations on the properties and behavior of some forms of collagen dissolved in dilute organic acids have been discussed. It is the biological implications which will concern us now. It was noted by the early French workers that tendon collagen which was soluble in dilute unbuffered organic acids in young animals became more insoluble with age. Orekhovitch and colleagues[31, 32] observed that the amounts of collagen extracted from animal skins with citrate buffers at acid pH diminished with age. This collagen fraction which could be readily precipitated as needle-like crystals and fibers they called "procollagen" implying precursor of the tissue fibers. With the exception of certain small differences[33, 34], the amino acid composition of "procollagen" is similar to collagen. The Russian workers also observed a more rapid rate of incorporation of C^{14} labeled glycine into "procollagen" than into the insoluble collagen, the latter being quite inert metabolically.

In 1951 Highberger, Gross and Schmitt[34] reported the extraction of collagen from fresh connective tissue with cold, mildly alkaline phosphate buffer and the reconstitution of this collagen fraction to typical fibrils *in vitro*. Harkness, Marko, Muir and Neuberger[35], using essentially the same procedure, subsequently demonstrated that this collagen fraction exhibited a relatively rapid rate of incorporation and "turnover" of C^{14} labeled glycine. Its metabolic activity was much higher than that of the collagen fraction extracted by acid citrate buffer, the "procollagen" of Orekhovitch. The remaining insoluble collagen of the skin was comparatively inert in terms of turnover. Gross, Highberger and Schmitt[36] then described the extraction of collagen from skin in cold salt solutions at physiological pH and at both high and physiological ionic strength (including serum ultrafiltrate), and showed that the collagen extracted thereby could be precipitated in the form of native fibrils simply by warming to 37°C; almost simultaneously Jackson and Fessler[37] reported similar observations. Jackson[38] then demonstrated again by isotope incorporation studies, that the neutral salt extractable collagen obtained from the actively growing carrageenin granuloma had a rapid "turnover" rate as compared to the citrate soluble and insoluble collagen fractions. Gross[39, 40] has since shown that the amount of neutral salt extractable collagen in the skin of guinea pigs is dependent on growth rate and is sharply reduced by several days of weight loss induced by fasting. After one or two weeks on a limited diet, which maintains static body weight, very little neutral salt extractable collagen can be obtained from skin. At least five days of renewed weight on an *ad lib* diet is required for the reappearance of this collagen fraction. The evidence is strong that the neutral salt extractable fraction is a newly synthesized collagen, probably produced less than 48 hours before extraction. Some of it may be in molecular dispersion in the ground substance and some in the form of loosely aggregated fibrils. The collagen isolated from such extracts, and purified, may be readily polymerized to typical fibrils simply by warming the solution in the test tube[36] (Plate III). It would appear that most of the extracted collagen molecules are complete and capable of association to form fibrils without further enzymatic modification or the intervention of any other substances, including mucopolysaccharides or glycoproteins—at least *in vitro*. It is of interest that the rate at which collagen fibrils will form *in vitro* by warming is strongly influenced

PLATE III—(From Gross, J. and Kirk, D., J. Biol. Chem. Reprinted by permission, ref. 41).

Fig. 1

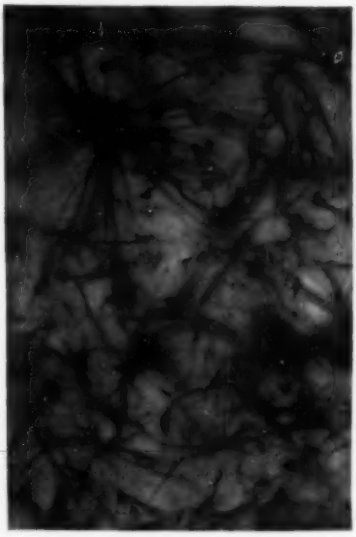

Fig. 2a

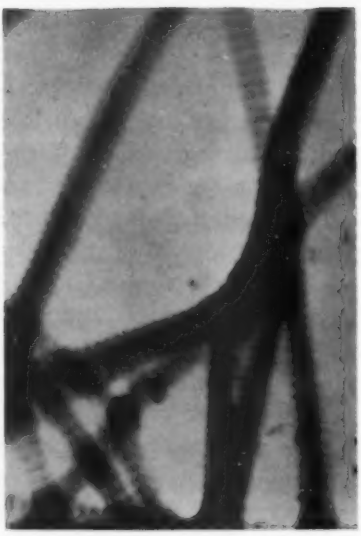

Fig. 2b

Fig. 1. Photograph of beaker of neutral phosphate solution of calf skin collagen and the rigid gel formed therefrom by warming to 37°C for 30 minutes.

Fig. 2a. Histological photomicrograph of gel stained with Mallory's aniline blue connective tissue stain, showing random orientation of fibers.

Fig. 2b. Electron micrograph of gel fragmented and stained with phosphotungstic acid. Magnification 17,200 X.

by the presence of low concentrations of small molecules such as urea, certain amino acids, small organic and inorganic anions and not obviously affected by hyaluronic or chondroitin sulfuric acids[27, 41]. Whether or not these observations can be extended to the intact tissues is yet to be demonstrated.

What, then, is the relationship between the collagen extracted in dilute acetic acid, in citrate buffer, in neutral salt solutions of different concentrations and the insoluble collagen? Do they each represent a slightly different molecular species in terms of composition or molecular configuration? In the opinion of this writer the small differences in amino acid composition observed may be the result of variable amounts of impurities having to do with the method of preparation. The observations that acid extracted collagen can be readily transferred to a cold neutral medium and remain dissolved—and vice versa—and will show similar behavior with regard to fibril formation, suggest that the molecules are essentially the same. This likelihood is further supported by the similarity of the measured dimensions of the collagen molecule (by physical chemical means) in the several media. There is the possibility, however, that one fraction of the collagen extracted from fresh skin with cold 0.14 *M* NaCl is different. Fessler[42] has separated the collagen of such extracts into three fractions, one of which, although it has the same molecular dimensions of the other two, will not form fibrils in the usual manner.

Some recent observations by the writer[43, 44] suggest that the differences between the several extractable collagens relate to the state of aggregation of the collagen molecules in the tissue and this in turn is a function of the time at which they were synthesized and aggregated into extracellular structures. Other factors may also be involved. It was observed that collagen fibrils recently formed from neutral solutions of purified collagen by warming to 37°C would redissolve on cooling. However, the longer they remained at the elevated temperature the more insoluble they became; within 24 hours of incubation, relatively little collagen would redissolve upon cooling. Collagen fibrils so formed at physiological ionic strength became insoluble more rapidly than those formed in hypertonic media. It was also observed that after a two week period of incubation at 37°C of similar preparations, the fibrils were no longer soluble in citrate buffer at pH 3.5 (Orekhovitch's medium).

Extrapolating back to the intact tissues these data suggest that the collagen which dissolved in cold neutral salt solutions at physiologic ionic strength was probably synthesized within several hours of extraction. That fraction removed at higher salt concentrations (ca, 0.45 *M* NaCl) had been synthesized within 1-2 days of extraction and the acid citrate extractable collagen may have been synthesized within 1-2 weeks of extraction. Thus, the major difference between the several soluble collagens would be their age. The mechanism of increasing insolubility may involve several factors, one of which is simply the increasing perfection of fit between collagen molecules as they gradually pack together into a more stable association in striated fibrils. Increasing numbers of secondary crosslinks would form (hydrogen bonds, electrostatic bonds between charged groups, Van der Waals forces) making disruption of the fibril increasingly difficult. The presence of other strongly polar and charged molecules, both small and large, perhaps including other proteins and mucopolysaccharides may aid in this process. However, this phenomenon can be observed to occur in preparations of highly purified collagen.

It seems likely that the diminished extractability of collagen in the aging animal[32, 45] is mainly a function of cessation of growth. Growth rate experiments described earlier suggest that collagen synthesis, at least in skin, ceases when weight gain ceases. The collagen, newly formed during the earlier growth phase, becomes increasingly insoluble with time by the mechanisms proposed above. One might speculate that if this phenomenon is a general occurrence when rigidly structured, sterically specific molecules are closely associated for prolonged periods of time, it may have broad significance with regard to the aging process.

To summarize, it seems likely that the fibroblast secretes the collagen molecules into the amorphous, extracellular ground substance and there, under the influence of the physiologic ionic atmosphere, temperature and perhaps certain rate regulating factors, the molecules aggregate spontaneously to form fibrils. There may, of course, be several earlier precursor forms, preceding the finished molecule, which have not yet been observed. The role, if any, played in this process by the non-collagenous components of the ground substance, such as mucopolysaccharides and other proteins, is still obscure. It is possible that these substances are important in the geographic ordering of the fibrils into bundles on the histological level or perhaps play some part

in regulating the rate of fibril formation and perhaps fibril size in the ground substance; but these are frank speculations at this time.

Wound Healing and Contraction

One of the oldest and still vigorously investigated problems in surgery is the mechanism of wound healing and the associated process of contraction. The early gross histological and tensile strength studies of healing wounds and the relationship of age, nutrition, species, etc. form a massive bibliography. In very recent years the broad applications of chemical methods have led to analyses of the sequence of chemical changes in healing and contracting wounds[46-48]. Most of this work has been based on the intuitively derived and generally accepted concept that changes in the central granulation tissues of an open wound result in a pulling together of the wound edges. The mechanism has been variously ascribed to diminution in total wound content, contraction of collagen, dehydration, and more recently by dynamic contraction resulting from contractile activity of the fibroblasts in the granulations.

In our laboratory the process of contraction in open skin wounds in guinea pigs has been reinvestigated by Grillo, Watts and Gross[49-51] both chemically and morphologically.

Full thickness excisions (including panniculus carnosus) were made on each flank of groups of guinea pigs and the rate of contraction in area and the linear movement of the edges measured daily. These were correlated with the total wet and dry weight of the granulation tissue within the wound edges, the water content, collagen (hydroxyproline), hexosamine, and non-collagenous protein (measured as tyrosine). Our daily measurements, in contrast to the earlier data[46-47], indicated a sharp and considerable rise in total wound tissue, wet and dry, and in collagen content between day 4 and 6, followed by as rapid a fall between days 6-7 and 10. This unexpected up and down fluctuation in wound content occurred during the period of maximum rate of contraction. Thus, there seemed to be no direct correlation between contraction and wound contents. This significant fluctuation was not observed in earlier studies probably because of the longer time intervals between observations.

This observation raised doubt concerning the direct role of the central granulations in bringing about approximation of the wound

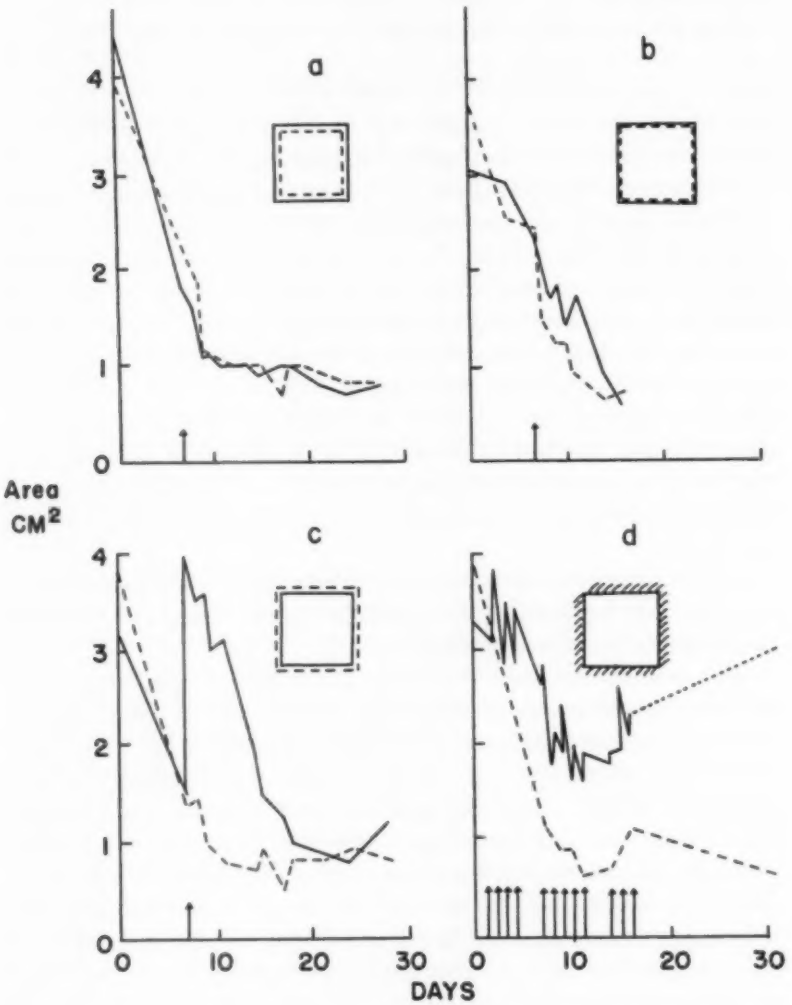

PLATE IV—Plot of change in area of excised square wound in guinea pig skin as a function of time in days. Dotted lines in graph represent contraction in control wound; solid lines represent course of contraction in manipulated wound. Solid line of square represents edge of original wound and dotted line of square represents edge of experimental excision after seven days of healing. Arrows indicate times of manipulation.

(a) Granulations excised to within a mm. of advancing wound edge.

(b) Granulations excised to advancing wound edge.

(c) Granulations plus one mm. of advancing wound edge excised.

(d) Repeated raising of edge of advancing wound margin.

edges. In a series of gross morphologic experiments the central granulations were completely excised within less than a millimeter of the wound edges during the period of maximum contraction rate. Excision was repeated as soon as new growth appeared. No change in either rate or extent of contraction of the wound was observed nor was there any distraction of the wound following excision as compared with control wounds in the same animal (Plate IV-a). Excision of granulations up to the advancing skin edges produced no interference in contraction rate (Plate IV-b). When one millimeter of the wound edge plus the granulations were removed, immediate large distraction of the edges occurred returning the wound to a size larger than the original area (Plate IV-c). Contraction then proceeded again at about the same rate as that of the initial wound. Removing the edge but leaving the central granulations intact had the same effect. In order to determine whether or not a sphincter mechanism in the wound edge was responsible, the edges of actively contracting wounds were separated from the central granulations then raised by sharp dissection from the base (Plate IV-d). Immediate increase in wound area occurred. If this was repeated on successive days, distraction occurred each time and the final area of the wound after 30 days was about the same or larger than that at the time of the first intervention. The inescapable conclusion was that the central mass of granulation tissue within the edges of the advancing skin was not necessary for contraction of an open skin wound. The machinery for contraction must reside in a very narrow "picture frame" of new tissue underlying the margin of skin at the edge of the wound, perhaps projecting slightly into the granulations. Histological and other studies are now in progress on the "picture frame". Our current hypothesis for the mechanism of contraction places the responsibility more on the cells in the wound margin rather than on some process involving the extracellular substances directly.

In summary, we have large masses of descriptive information concerning the surface appearance and behavior of diseases thought to involve connective tissue, but very little knowledge which leads to an understanding of mechanisms. One hopes that a better understanding of the fundamental nature and behavior of connective tissues, their composition, structure, synthesis, degradation and function may lead to fruitful directions of study into the pathogenesis of diseases affecting them.

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TENTH ANNIVERSARY MEETING

SECTION ON MICROBIOLOGY

January 15, 1958

Symposium on

RECENT CHANGES IN THE MANAGEMENT OF TUBERCULOSIS

SOME IMPLICATIONS OF TUBERCULOSIS TO-DAY

J. Burns Amberson

Professor Emeritus of Medicine, College of Physicians and Surgeons, Columbia University; Consulting Physician, Bellevue Hospital, New York, N. Y.

THERAPEUTIC FAILURES IN PULMONARY TUBERCULOSIS

John H. McClement

Associate Professor of Medicine, College of Physicians and Surgeons, Columbia University; Visiting Physician-in-Charge, Chest Service, Bellevue Hospital, New York, N. Y.

RAPID DETECTION OF DRUG RESISTANT TUBERCLE BACILLI BY SLIDE CULTURES OF SPUTUM

Cynthia H. Pierce

Rockefeller Institute for Medical Research

HUMAN TRIALS OF TUBERCULOSIS PROPHYLAXIS

Mrs. Shirley H. Ferebee

Tuberculosis Program, U. S. Public Health Service, Department of Health, Education, and Welfare, Washington, D. C.

Beatrice C. Seegal, *Chairman* Harry M. Rose, *Secretary*

SOME IMPLICATIONS OF TUBERCULOSIS TO-DAY

THE INFLUENCE OF CLINICAL TREATMENT*

J. Burns Amberson

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It is a privilege to be opening this symposium of the Section on Microbiology of the Academy, especially on the occasion of the tenth anniversary of the Section. Scarcely more than ten years ago we became convinced that the pot of gold at the end of the rainbow, which for centuries had lured men on in their search for a specific therapeutic agent against tuberculosis, was no illusion. Since then mankind has shared in the riches. In medicine the microbiologists through their studies of the tubercle bacillus in relation to chemotherapy have enhanced its status as one of the most interesting and protean weeds which plague the animal species, while the clinicians have formulated microbiologic problems which can be identified and properly rated only by practical bedside experience. Happily, there has been a meeting of the minds all along the way, and it is gratifying to see it exemplified in this evening's meeting.

In the ten years since the use of specific chemotherapy for tuberculosis became almost universal, changes in many aspects of the problem have come very swiftly, necessitating a revision of the principles and methods of its solution. The death rate from the disease in the United States (estimated as 8.5 per 100,000 population in 1956) declined during these ten years four times more rapidly than it did during any single decade in the early part of the century. Specific therapy must have been responsible for much or most of this acceleration. The death rate today is about 4 per cent of that prevailing in 1900, and our country, along with others such as Denmark, Holland, Sweden and Canada, has been characterized by Etienne Bernard as one in which tuberculosis is no

* Presented at the *Tenth Anniversary Meeting* of the Section on Microbiology at The New York Academy of Medicine, January 15, 1958.

longer to be regarded as a social menace. This view, of course, depends on the definition one chooses for the term. In a comparative sense, as Bernard intends, it is acceptable when one considers death rates of 85 in Calcutta, 108 in Hong Kong and 250 in Korea. There, and in many other parts of the world, the shadow of tuberculosis hovers over families and households as it did here in the days of our forefathers. The progress here in recent years has diminished the menace, but nevertheless has left some difficult situations and potentialities still to be faced. For example, striking geographic differences in death rates are reported in our country by the U.S. Public Health Service, varying, in 1956, from 2.6 per 100,000 population in North Dakota to 18.9 in Arizona. In New York City, where the rate in 1957 was 13.2, the range was from 5.6 in the Borough of Richmond (Staten Island) to 25.1 in the Borough of Manhattan, and there was a nearly proportionate variation in the number of new cases reported, viz., 29.1 per 100,000 in Richmond up to 167.9 in Manhattan. This five- to six-fold difference, generally charged to varying standards and conditions of living, reveals a social menace, the degree of which varies according to the base line which might be designated as zero. An important factor obviously is the opportunity in a given locality for the transmission of infection, and this too varies widely. Among large groups of recruits in the United States Navy, aged 17 to 20, reactions to the skin test with tuberculin have been elicited recently in approximately 5 per cent, whereas in other parts of the world, such as Japan, the percentage of reactors at this age is reported to be closer to 70 or 80 per cent. Among students of secondary and high schools in New York City, fragmentary statistics reveal a variation of probably 5 to 30 per cent. Since the infection is transmitted usually from the person with cavitary pulmonary tuberculosis and a "positive" sputum, it is also obvious that the number of tuberculin reactors, especially among young people, is a reflection of the number and proximity of such pulmonary cases existing in the community, and that this in turn is related to the effectiveness of the means of discovering the disease and the adequacy of therapy. Chemotherapy is emphasized because this has the greatest potential of arresting tuberculosis before it becomes cavitary and communicable and of reducing or eliminating communicability after the cavity has developed. All other measures, except the segregation and education of the patient, play a subsidiary role in this respect. While it is difficult to prove the precise connection of cause and effect,

it is safe to assume that the availability and proper application of chemotherapy in many parts of the world has had a strong influence in drying up sources of infection and reducing its transmission. It is theoretically possible in the future to extend treatment so that the circle of infection and disease could be broken, but there are hindrances, and, before these can be overcome, they must be identified and evaluated.

A great difficulty which still baffles us is the extent and cavitary character of pulmonary tuberculosis when it is first diagnosed and presented for treatment. This, of course, is due largely to the mild and insidious course of the disease in its early stages. The situation has improved somewhat, owing to the initiative of physicians and public health agencies to ferret out the unknown cases. In the hospital now one does not observe the number of severe secondary complications, such as tuberculous laryngitis and enteritis, which once existed, often foreshadowing early death. In fact the incidence of these was declining slowly before the days of chemotherapy. But it is still true that the majority of cases discovered in organized surveys of segments of the population are already in an advanced cavitary stage. Another impressive observation to-day relates to the resolution of exudative elements of tuberculous lesions. This important manifestation of subsiding infection has always been recognized as an early sign of rising vital resistance against invading tubercle bacilli and of the salutary effects of treatment. Under the influence of rest and other general measures, resolution was often striking but in many cases signified only a temporary and dubious gain. The element of precariousness still exists; nevertheless, in general, resolution is now seen to occur in greater degree and with greater frequency and rapidity. This is credited largely to chemotherapy but also testifies to some success in bringing more patients to treatment before the greater part of their disease has become permanently established by the processes of necrosis and fibrous organization. Accordingly, the pathological character of lesions, as well as their anatomical extent, assumes greater importance as a factor in the efficacy of treatment of the patient and in breaking the chain of transmission of infection to others. The mere designation of a far advanced stage and bilateral distribution of pulmonary tuberculosis does not have the fullest meaning. If this also connotes caseation, cavity formation and fibrosis, the implications are clear, although not as hopeless as they once were. But if the lesions are composed mostly of exudative and still living cellular elements, the

outlook for recovery under proper treatment is usually very favorable. The latter condition is often the result of an extremely rapid or even explosive spread of the infection through the lungs as the early limited lesion, which may have been symptomless, undergoes liquefactive necrosis and excavation.

The varying tempo of tuberculosis, especially in its early phases, defines in part a problem which must be met if the advantages of present knowledge are to be realized fully. And, incidentally, this recalls to mind the wisdom of Thomas McCrea who said, a generation ago, that an important duty of the physician is to make an early diagnosis of advanced tuberculosis.

The optimism which one may feel about the advanced case of comparatively recent development is based partly on the observation that many of such, especially in young people, are of a pneumonic character and predominantly unilateral in distribution. Under chemotherapy the extent of resolution is sometimes phenomenal. Younger lesions in the less affected lung may leave only minimal residues which usually go on to dependable healing, and caseous remains and cavities in the originally diseased lung may then be resected surgically if this proves to be necessary. Such cases usually are more successfully manageable than more chronic disease of comparable extent and distribution, because in the latter there is more scarring and other irreversible change. While a patient is unfortunate not to have his tuberculosis discovered in a smaller and more limited stage, this need not be an unmitigated disaster. Lengthy and good treatment will often prevent his drifting into the chronic fibroid cavitary phase—which too commonly was the compromise to be made with death when the rest cure was the only defense—and, what is more, he has a better chance to stay well without being an infectious menace to his associates. There is much to be gained, therefore, in continuing to look for the unknown case.

But the fact remains that the best prospect of permanent recovery from tuberculosis rests in its early diagnosis and prompt treatment when it first appears as a small lesion with little or no cavity formation. Even before the coming of specific chemotherapy the prognosis under treatment was excellent, but frequently at a cost of lengthy periods of confinement in a hospital or sanatorium and strict adherence to a regimen of rest. In these cases there is good reason now to believe that the length of treatment can be shortened substantially without added risk, and the

evidence indicates that within five to ten years—the period of follow-up which is now a matter of record—the incidence of relapse in the properly treated case is almost nil. As a consequence these patients seldom, if ever, transmit the infection.

What is proper treatment to-day is frequently a matter of judgement—and sometimes of poorly supported opinion. Comprehensively organized experiences, such as those of the Veterans Administration-Armed Forces Hospitals, the United States Public Health Service and the British Research Council, together with more limited intensive studies, have contributed uniquely to the advancement of our knowledge in this direction, and certain principles have become well founded. Regimens of chemotherapy, which are very dependable up to a point, have been defined. It has been demonstrated that in most overtly active cases short periods of such treatment are inadequate and a poor defense against relapse. The administration of drugs for a year or more after the disease has come under satisfactory control is deemed essential, and in many cases the time is greatly prolonged. The continuation of a regimen without unnecessary interruption during this time is accepted as an important principle. Prompt detection of the development of bacterial resistance to the drugs in use is desirable in many cases in which an arrest of the disease has not yet been achieved and a change in regimen may be indicated. The time demanded for this determination—as much as several months—and the technical difficulties may leave the fire of tuberculosis burning too long before the appropriate repellent is identified and applied. Consequently the physician is often forced to rely on his clinical findings. Fortunately these, if carefully assembled and soundly judged, are often very dependable, but in certain critical situations they are misleading. A more rapid method of determining bacterial resistance to drugs is much to be desired and we will learn with interest this evening of the efforts of Doctor Pierce in this direction.

In the advanced active case of tuberculosis there is no disagreement about the wisdom and need, not only of chemotherapy, but also of rest and various adjuvants. But even here, the question, How long rest? remains unanswered except perhaps in the mind of the physician who has a conviction based on long experience or in the mind of the patient who, for reasons of his own, decides to forego it. In the less advanced, more hopeful cases, a number of studies, such as those of d'Esopo, Weir

and Hirsch and their co-workers, indicate that under chemotherapy the early results of treatment are just as good with "modified" rest as they are with strict bed rest. All of us have been impressed by the excellent response of certain patients who have relied solely on chemotherapy and who have never reclined on a bed except at night; and we should remember that such things also occurred—though much less often—in the pre-chemotherapeutic days when some patients had a natural or acquired immunity to our wisdom and advice. Whether or not it will ever be possible to determine by some scientifically accurate criterion when rest is indicated, how much, and how long, remains to be seen. No one knows, for instance, in the case of the recently developed pneumonic lesion, about 1 or 2 cm. in diameter, in an adolescent girl, just what may be the biochemical and circulatory conditions in and about the lesion which favor its early partial resolution and permanent healing, and in what manner this is promoted, as I believe, by rest treatment alone. Nor do we know exactly why, after a period of months or years, the inspissated caseous remains of these lesions—large or small—sometimes liquefy and initiate a relapse of the disease; certainly chemotherapy alone does not uniformly and completely disrupt this mechanism, whatever it may be. In some human bodies, however, in which obsolete sterile lesions are found, nature alone seems to have accomplished the disruption. Obviously, we need to know more about the mechanisms of natural resistance against tuberculosis and the conditions under which they may operate most effectively. Until then rest treatment will be prescribed on an empirical basis.

These observations—and there are many more which might be mentioned—are a few reflections not only of the enormous increases of our knowledge in recent years but also of problems, not altogether new though somewhat better defined, which are of great scientific interest and of the utmost importance to the health and welfare of people everywhere.

THERAPEUTIC FAILURES IN PULMONARY TUBERCULOSIS*

JOHN H. MCCLEMENT

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UNTIL quite recently the over-all results of the treatment of pulmonary tuberculosis have been very poor. During most of the first half of this century the case fatality rate did not change appreciably and death from progressive disease or continued disability was the expected fate of all but a few tuberculous patients. The discovery of effective antimicrobial drugs and the development of finer thoracic surgical techniques have greatly altered this grim picture.

Carefully controlled studies on large groups of patients have established principles for the use of our presently available therapeutic modalities in the initial treatment of patients with pulmonary tuberculosis which are now almost universally accepted. A few details of what currently constitutes optimum therapy in various groups of patients remain to be worked out. It is generally agreed that patients undergoing initial treatment for tuberculosis should be given a chemotherapeutic regimen that includes isoniazid, and that streptomycin or para-aminosalicylic acid (PAS) or both, should be given concurrently and daily with the isoniazid. This regimen should be continued without interruption for at least a year and often for an even longer period. At an appropriate time during this course of treatment, irreversible and potentially dangerous residual lesions should be identified and if it is feasible, these should be excised.

Numerous exceptions, elaborations, or amendments to this somewhat over-simplified statement of principles may be justified by presently available clinical and laboratory studies. Some investigators would insist that the dose of isoniazid is critical and that either all patients should be tested to identify those individuals who inactivate isoniazid readily or

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that a high dosage of isoniazid should always be employed. The excellent results that have been reported by many clinicians who have used relatively small doses of isoniazid suggest, however, that if a high dosage of isoniazid is important, this is the case in only a very small segment of our patient group. Other observers might suggest that combined therapy is not always necessary and that patients can be selected in whom isoniazid alone is adequate therapy. The place of rest, adrenocortical steroids, triple drug therapy, and daily versus less frequently administered streptomycin could also be introduced. In considering surgical therapy a vigorous debate could be organized as to what constitutes a dangerous or irreversible residue, which residual lesions should be resected, and the place of collapse therapy in a therapeutic program.

In spite of the healthy differences of opinion which this listing indicates, experience shows that the treatment of pulmonary tuberculosis is becoming increasingly standardized and effective. Successful therapy of advanced pulmonary tuberculosis is now so common and so consistently to be expected that it may be fruitful to examine the reasons for failure when this occurs.

Death, continued chronically active disease, relapse, and respiratory insufficiency after the active disease has been controlled, are some of the categories into which therapeutic failures in tuberculosis can be divided.

Death—Vital statistics indicate that the death rate from tuberculosis is declining in nearly all parts of the world where reliable data can be collected. In the last decade a fall in the case fatality rate, which is most often due to improvements in therapy, has also been widely observed. During this same period there has been a steady decrease in the number of patients admitted to tuberculosis hospitals, who died in the hospital. While such data indicate a steady decline in the number of deaths only a review of individual patients dying with tuberculosis can yield qualitative information about such cases.

The case records of one hundred consecutive patients who died on the Chest Service of Bellevue Hospital after the diagnosis of active tuberculosis had been made, have been reviewed. This group includes all such deaths observed on this Service during part of 1954 and all of 1955. Some of the data from these cases are presented in Table I. It can be seen that nearly all of these patients were men, and that the majority were over 50, had not previously had tuberculosis diagnosed or treated

TABLE I—OBSERVATIONS IN 100 PATIENTS DYING WITH ACTIVE PULMONARY TUBERCULOSIS

Male	87
Over 50 years of age	60
Tuberculosis not previously diagnosed	51
No previous chemotherapy for tuberculosis	57
Died in 28 days or less after admission	64
Principal causes of death:	
Non-tuberculous disease	29
Tuberculosis and non-tuberculous disease	28
Tuberculosis	43

and died during the first four weeks after admission.

In 29 of these patients, a review of the record clearly indicated that the principal cause of death was not the co-existing active pulmonary tuberculosis. Among this group were 17 patients with malignant tumors, three with severe vascular disease, four with advanced pulmonary emphysema, four who died of operative or postoperative complications, and one patient with a bleeding peptic ulcer.

In 28 patients it was not possible to unequivocally assign the principal cause of death either to active pulmonary tuberculosis or to the other serious complicating disease which was present. In this group, cases of uncompensated cirrhosis, uncontrolled diabetes, as well as carcinoma, emphysema, surgical accidents and vascular disease were encountered.

In 43 patients far advanced pulmonary tuberculosis clearly seemed to be the principal cause of death. While most patients in this group were debilitated, had evidence of malnutrition, and sometimes dehydration, evidence of other significant organic disease was not detected. Data from this sub-group are presented in Table II. The same characteristics observed in the whole group dying with tuberculosis were observed in this sub-group. It is composed mainly of men over 50 years of age who had not been previously diagnosed or treated. Only seven patients in this group died after the first 28 days in the hospital (30, 31, 32, 66, 110, 111 and 125 days), and three of these deviated from this period by only a few days. While treatment of this group was not standardized or

TABLE II—OBSERVATIONS IN 43 PATIENTS DYING WITH ACTIVE PULMONARY TUBERCULOSIS AS THE ONLY MAJOR CAUSE OF DEATH

Male	38	(88%)
Over 50 years of age	28	(65%)
Tuberculosis not previously diagnosed	31	(72%)
Tuberculosis not previously treated	37	(86%)
Died in 28 days or less after admission	36	(84%)

uniform, all of the generally used supportive measures were available and were employed as indicated. Transfusion, fluid replacement, and support of the blood pressure with sympathomimetic drugs were all commonly employed. Adrenocortical steroids were also used in the treatment of some of these patients. The most frequently employed chemotherapeutic regimen was streptomycin and isoniazid. Some patients were treated with PAS as well. It seemed possible that the presence of drug resistant organisms might have contributed in part to the fatal outcome in five of these 43 patients.

While certain local conditions that influence the selection of patients admitted to and treated at Bellevue Hospital may have affected the character of these observations, it seems probable that our experiences with patients dying of pulmonary tuberculosis are similar to that of many others elsewhere in the United States at this time. Our experiences suggest that in our community death from tuberculosis, *per se*, is unusual. Such deaths occur mostly in older individuals who for some reason avoid medical diagnosis or treatment until they are nearly moribund. Even in this group a fatal outcome can usually be avoided if a supportive regimen can be organized which is adequate to keep the patient alive for the first few weeks. These observations also illustrate again that tuberculosis is coming to be more and more a disease of the older age groups in whom other complicating diseases are to be expected. The very small number of patients in this series who died from tuberculosis after prolonged chemotherapy suggests that even when such therapy does not arrest the disease, it so alters its progressive character that death is an infrequent consequence. This finding may be an important consideration when one must decide whether to use surgical treatment in patients who are poor operative risks, because of ad-

TABLE III—OBSERVATIONS IN 50 CASES RETREATED FOR TUBERCULOSIS

Males		40
Over 50 years of age		11
Extent of Disease:	*Initially*	*At time of retreatment*
Far advanced	18	23
Moderately advanced	10	19
Minimal	5	6
Extrapulmonary tuberculosis only	3	2
Unknown	14	
Reason for terminating initial treatment:		
Irregular medical discharge		26
Discharged from treatment by physician		22
Unknown		2

vanced age, complicating non-tuberculous disease, or respiratory insufficiency.

Continued chronically active disease and relapse. These manifestations of incompletely effective initial treatment are frequently difficult to distinguish and will be considered together here. Patients admitted for the retreatment of pulmonary tuberculosis provide an opportunity to study retrospectively the factors which may contribute to these two categories of treatment failure.

Dr. Julia M. Jones* has reviewed 50 randomly selected patients admitted to the Chest Service, Bellevue Hospital, who required retreatment for tuberculosis. These patients were selected from the same 1954-1955 period as the group of patients in the previous section that died, and no patient that died is included in this group. All had had treatment interrupted for at least one month at the time of admission and most for much longer periods. Because initial treatment had been carried out in many different places and included a variety of different chemotherapeutic regimens, this group is probably representative in many ways of the current problem among patients who require retreatment.

* Jones, Julia M. Retreatment of tuberculosis. Presented at Eastern Section, American Trudeau Society meeting, October 19, 1957, Atlantic City, New Jersey.

Some of the data from this group are presented in Table III. These patients were also found to be predominantly male, but the average age was lower than in the previously considered groups. Far advanced disease predominated, both initially and at the time of retreatment. In the majority (26) of these patients initial treatment had been terminated without medical sanction. In the other 24 patients, it was possible from such information as could be obtained, to identify 11 patients in whom active disease was present when treatment was stopped. In the remaining 13 patients it was frequently difficult to judge whether readmission resulted from a true relapse or from chronically active disease which had never been arrested by initial treatment. Clearly, however, chronically active disease from incomplete initial treatment, rather than relapse, is the principal cause of retreatment in this series. In fact, cases demonstrating true relapse in whom initial treatment was prolonged and uninterrupted, and in whom cavitary residues could not be demonstrated at the conclusion of treatment are so infrequently encountered that it is difficult to define the characteristics of such a group.

A review of patients in this group revealed many stigmata that suggested that severe emotional maladjustments had interfered with initial treatment. Fifteen of these patients were severe alcoholics, seven had histories of major emotional disorders, and 26 had a history of one or more irregular medical discharges. The difficulties attendant on treating such patients are well known to most physicians who work with tuberculous patients. The problem of how to adapt our therapeutic program to meet the needs of this heterogeneous group of disturbed people is probably the most important problem remaining unsolved today in the treatment of tuberculosis in this country. Attempts to make these patients adapt to our presently available treatment regimens have certainly had very limited success.

While it is not the purpose of this presentation to discuss the treatment of this difficult type of patient, in whom treatment has already failed one or more times, it can be said that in this series of patients, reinstituted therapy usually resulted in the disease again being brought under control. Chemotherapy, often with the less commonly used antibiotics, and resectional pulmonary surgery, seemed to completely control the pulmonary tuberculosis in many of these individuals, and in others to control it sufficiently for them to resume their program of irregular medical discharges.

Respiratory insufficiency after completed treatment. While this type of therapeutic failure has not been studied in a systematic fashion, scattered observations on individual cases indicate that while far advanced disease must have been present in all such cases, other factors may also be important in causing disabling respiratory insufficiency. Patients have been observed with very far advanced pulmonary tuberculosis who after the disease has been completely arrested have only minor changes in lung volumes and ventilatory function. At the same time other patients have been observed who are left with complete disability and all the clinical and physiologic findings of advanced pulmonary emphysema. In this latter group there is often either a history of multiple very fine, widespread bronchogenic disseminations of the type seen with tuberculous laryngitis or repeated untreated hemoptyses, or suggestive evidence that pulmonary emphysema unrelated to the tuberculosis has developed simultaneously.

In conclusion, our experience indicates that most therapeutic failures in pulmonary tuberculosis can usually be attributed to one of the following: far advanced disease at the time of initial discovery; inability of the patient for social or psychological reasons to accept a complete course of treatment; failure of the physician to administer adequate chemotherapy or to see the need for adjunctive surgery; and the presence of other complicating diseases.

RAPID DETECTION OF DRUG RESISTANT TUBERCLE BACILLI BY SLIDE CULTURES OF SPUTUM*

CYNTHIA H. PIERCE

Rockefeller Institute for Medical Research

WITH the widespread use of antimicrobial therapy in tuberculosis, there is urgent need for early detection of the emergence of drug resistant strains of tubercle bacilli. The methods most generally employed for the determination of susceptibility of Mycobacteria to antimicrobial agents require long periods of time—often as long as two months. For this reason in many instances, as Dr. Amberson has already stated, the physician finds it necessary to change drug therapy before laboratory results are available.

Slide culture techniques have shortened appreciably the time required for the detection of growth of tubercle bacilli. A paper by Reed and Morgante[1] summarizes their own investigations and provides an extensive review of the literature dealing with such methods. These techniques are being used for drug susceptibility determinations in foreign countries, but have received relatively little attention in the United States.

I should like to discuss some results obtained from slide cultures of tuberculous sputum and to emphasize in particular the advantage of this method for the early detection of drug resistant organisms.

The procedure being used at present in our laboratory is a slight modification of that previously reported[2].

For the protection of personnel all manipulations with the exception of the homogenization of infectious material are carried out in a hood such as illustrated in Figure 1. This is kept under negative pressure and is equipped with ultraviolet light to provide sterilization of the interior chamber before and after slide cultures are made.

Sputum specimens are collected over a 24 hour period. In patients

* Presented at the *Tenth Anniversary Meeting* of the Section on Microbiology at The New York Academy of Medicine, January 15, 1958.

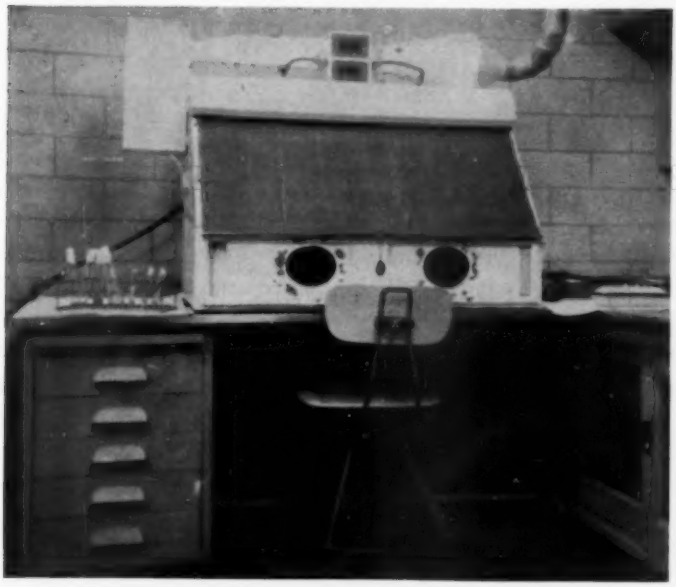


Fig. 1—Safety cabinet*, **

* Designed at the Rockefeller Institute for Medical Research.
** Shade pulled over glass surface when ultraviolet lights are on.

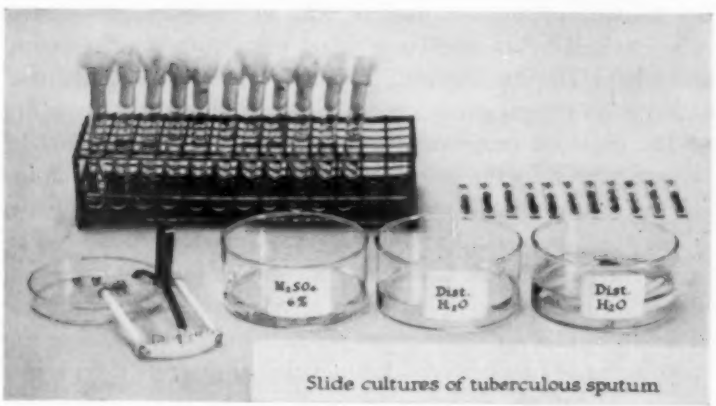


Fig. 2—Steps in the preparation of slide cultures.

receiving drug therapy all antimicrobial agents are withheld for a period of 24 hours preceding the sputum collection as well as during the 24 hour collection period. Homogenization of the specimen is accomplished by shaking the original container in a paint shaker for one to three minutes. The different steps involved in the preparation of the cultures are illustrated in Figure 2. Smears of the homogenate are made on a series of small glass slides—the number being determined by the different antimicrobial agents under test. These slides are merely the standard microscopic slides cut longitudinally into three strips.* The smears are left to dry in Petri dishes at room temperature after which they are placed in a teflon rack** which can hold a maximum of 16 slides. (Teflon has the advantage of being a plastic easily tooled, is inert in strong chemical solutions, and can withstand autoclaving.) The filled rack is then immersed in 6 per cent sulfuric acid and left for six minutes —a time adequate to destroy contaminating microorganisms other than tubercle bacilli in the sputum. The acid is removed by two separate rinses in sterile distilled water contained in crystallizing dishes. The slides are subsequently removed from the racks by means of forceps and deposited without drying into Wassermann tubes—one containing the control medium only (Dubos basal liquid medium plus oleic acid albumin), the remainder of the series containing five-fold dilutions of the drugs to be tested. The tubes are incubated at 37° C. in an upright position for five or six days after which the slides are removed and allowed to dry at room temperature. After fixation with absolute methyl alcohol, they are stained by an acid-fast method using pararosanilin instead of basic fuchsin. The staining with carbol-pararosanilin is carried out for ten minutes on a metal staining rack to insure killing of the viable organisms. The slides are then ready for microscopic evaluation.

Figure 3 presents the gross appearance of a set of slide cultures in which the tubercle bacilli are susceptible to isoniazid and streptomycin. It is obvious that marked multiplication of tubercle bacilli has taken place on the control slide C—i.e., the one grown in medium containing no drug. No multiplication has taken place on the slides grown in the presence of 1, 5, or 25 micrograms per milliliter of isoniazid or similar concentrations of streptomycin. Examination of these six slides under oil immersion reveals the presence of bacilli which were in the original

* Obtained commercially from Scientific Glass Apparatus Co., Inc., 100 Lakewood Terrace, Bloomfield, New Jersey.

** Designed by Mr. Nils A. Jernberg, Instrument Shop, Rockefeller Institute for Medical Research.

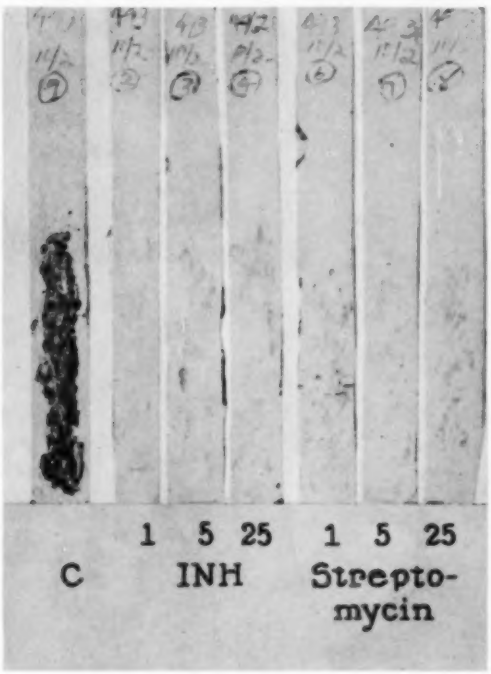


Fig. 3—Slide cultures of tubercle bacilli susceptible to isoniazid and streptomycin*.

C=control medium without drug.

1, 5, 25=final concentration (microgram per ml. of medium) of indicated drug.

*=Standard microscopic glass slides cut by hand before commercial product became available.

smear occurring singly or in small groups of four to five organisms. In contrast, examination of the control slide shows extensive multiplication with the typical morphology characteristic of virulent tubercle bacilli. This can be seen in the photomicrographs, Figures 4a and 4b. Tubercle bacilli arranged in parallel and forming the typical long, winding, cord-like pattern are seen in the control slide culture (Figure 4a). No multiplication of organisms is observed on slide cultures grown in the presence of isoniazid or streptomycin which are represented in Figure 4b.

Not all sputum specimens are as heavily positive for acid-fast bacilli

Fig. 4a—(Magnification 1315 X)

Multiplication of tubercle bacilli in control medium without drug.

as the one shown in Figure 3. The range which may be expected is illustrated in the photomicrograph (Figure 5). These are typical areas at low power magnification (124x) of slide cultures from sputa of three patients. It can readily be seen, however, that even when relatively few organisms are present in the inoculum (Figure 5-c), multiplication can be detected within seven days.

The slide culture technique for testing drug resistance is generally applicable only to specimens in which bacilli are demonstrable by direct smear. Tables I and II serve to demonstrate this point.

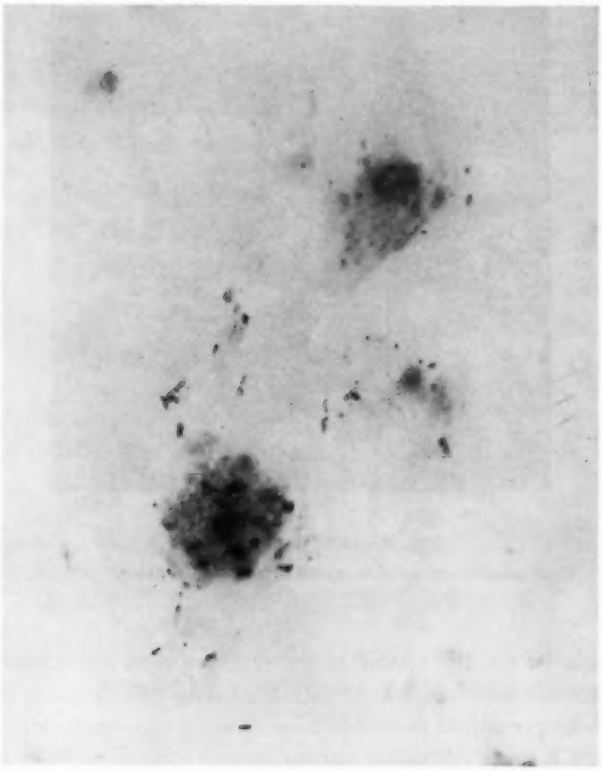
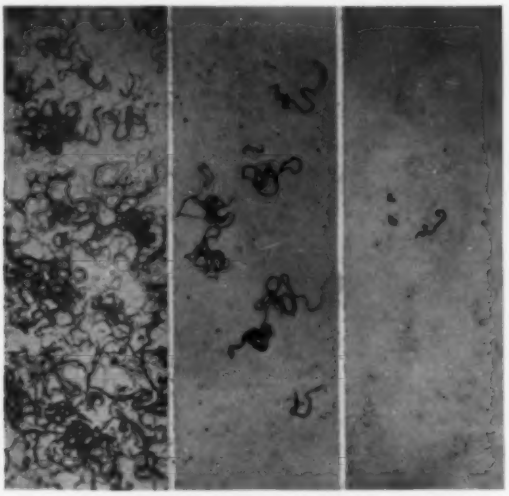


Fig. 4b—Appearance of tubercle bacilli present in original smear but non-multiplying in the presence of isoniazid or streptomycin.

Table I presents results obtained from a series of sputum specimens which were positive for acid-fast bacilli by direct smear. Three specimens were obtained from each patient at intervals of two weeks. After the sputum was smeared for slide culture, it was then concentrated in a routine manner by NaOH digestion, centrifugation and neutralization with HCl. The sediment obtained was washed and recentrifuged before inoculation onto egg slants (Löwenstein-Jensen medium). All slide cultures were examined after an incubation period of five days and all showed multiplication of acid-fast bacilli (microcolonies of tubercle bacilli could have been detected as early as three days). The figures in



a. b. c.

Fig. 5*—(Magnification 124 X)
Positive slide cultures of sputa from three different patients.

the column on the right indicate the incubation time required for the appearance of visible colonies of tubercle bacilli on egg slants. Not only is a much longer period required—even up to 42 days in the case of L. S.—but there is great variability among sputum specimens from the same patient in all instances. This was not observed when slide cultures were used.

Table II presents comparative results obtained by slide culture and egg slant inoculation when the sputum specimens were negative for acid-fast bacilli by direct smear. It can readily be seen that all the slide cultures were negative. One must bear in mind, however, that the inoculum deposited on the egg slants was approximately 20 times that used for slide cultures—therefore, it is not surprising that the slide cultures are negative when only a very few colonies of tubercle bacilli (less than 10) are recovered from sediments of sputum. (Sediment obtained after NaOH digestion of sputum does not adhere to slides during the process of slide culturing.)

* Previously published in *American Review of Tuberculosis and Pulmonary Diseases* ref. 2. Reprinted by permission.

TABLE I—GROWTH OF TUBERCLE BACILLI FROM SPUTA*
(POSITIVE BY SMEAR)

Patient	*Time (in days) required for detection of growth*	
	Slide culture	*Egg slant***
JM	5	19
	5	30
	5	40
CT	5	25
	5	12
	5	17
JMa	5	12
	5	18
	5	18
LS	5	24
	5	42
	5	21
MV	5	21
	5	18
	5	21

* Condensation of results previously published[2].
** More than 100 colonies or confluent growth on all slants.

TABLE II—GROWTH OF TUBERCLE BACILLI FROM SPUTA*
(NEGATIVE BY SMEAR)

Patient	*Slide Culture*		*Egg Slant (inoculated in triplicate)*	
	Day of observation	*Growth*	*Day of detected growth*	*No. of colonies per slant*
GG	8	Neg.	17	2, 3, 5
	5	"	33	1, 1, 0
	5	"	41	1, 0, 1
MB	5	"	58	1, 0, 0
	5	"	50	9, 7, 0
JM	5	"	21	1, 0, 0
ET	5	"	23	8, 3, 5
BB	5	"	25	10, 2, 4

* Results previously published[2].

TABLE III—DRUG RESISTANCE OF TUBERCLE BACILLI FROM SPUTA*

Patient	Time (in days) required to assess resistance														
	Slide Culture									Egg Slant					
	Control no drug	Isoniazid micrograms/ml.				Streptomycin micrograms/ml.				Control no drug	Isoniazid micrograms/ml.		Streptomycin micrograms/ml.		
	0	1	5	25	125	1	5	25	125	0	1	5	10	100	
JL	5	—**	—	—	—	—	—	—	—	19	—	—	—	—	
MB	5	5	5	5	—	—	—	—	—	19	19	19	—	—	
BN	6	6	6	6	—	—	—	—	—	11	30	30	—	—	
MV	5	—	—	—	—	5	5	5	—	18	—	—	23	23	
CT	5	—	—	—	—	5	5	—	—	17	—	—	38	—	
CT	5	—	5	—	—	5	5	—	—	12	40	—	53	—	
LS	7	7	7	7	—	7	7	7	7	46	46	46	46	46	

* Condensation of results previously published[2].
**—Negative (no multiplication).

A comparison of the two methods for the determination of resistance of tubercle bacilli to isoniazid or streptomycin is illustrated in Table III. Four concentrations of isoniazid or streptomycin were used in the medium for slide cultures, and the two standard concentrations of isoniazid or streptomycin in the egg medium. This chart presents results of slide cultures stained at five, six or seven days of incubation. Later readings of duplicate sets stained after two weeks' incubation gave identical results as far as resistance to the antimicrobial agents is concerned. It is immediately evident that earlier detection of growth of tubercle bacilli was obtained from all specimens by the slide culture technique as compared with egg slants.

Sputum of the first patient, J. L., yielded acid-fast organisms which were sensitive to concentrations of isoniazid or streptomycin as low as 1 microgram/ml. In contrast, the organisms from M. B. were resistant to 25 micrograms of isoniazid but sensitive to streptomycin. In the case of B. N., who also had organisms resistant to 25 micrograms of isoniazid, this fact was not obtained by the egg slant method until almost three weeks after the control culture had grown, 11 days vs. 30—whereas by slide culture the organisms grew in the presence of isoniazid at the same rate as the control. The organisms from M. V. were resistant to strepto-

mycin and again the lag in determination of resistance can be noted with the egg slants. The same holds true for the first specimen from C. T. With a specimen obtained from C. T. two weeks later, the development of resistance to isoniazid as well as to streptomycin is noted by slide cultures within five days but with egg slants resistance to isoniazid was not apparent until 40 days of incubation, resistance to streptomycin not until 53 days. The last patient, L. S., had organisms highly resistant to both isoniazid and streptomycin—the endpoint of streptomycin resistance not being obtained. Moreover, a positive culture was not detected on the control egg slant or drug tubes until 46 days. This is in striking contrast to the results obtained within seven days by slide cultures.

Discussion and Summary

The over-all results in terms of resistance or susceptibility of tubercle bacilli to various concentrations of isoniazid or streptomycin were essentially the same with either the slide culture method or the egg slant inoculation. However, the difference in time required for the determination is quite striking—one week or less with slide cultures as contrasted with three to six weeks with egg slants. It should be re-emphasized also that with slide cultures resistant organisms were demonstrable as soon as growth was determined on the control slide, whereas in many instances there was a great delay in the appearance of resistant colonies on egg slants even after the control tube was positive.

Slide cultures of tuberculous sputum have the following advantages over the routine method of solid media inoculation:

1. Foremost, the resistance of tubercle bacilli to antimicrobial agents can be detected at an early date,

2. The time-consuming procedure of digesting, centrifuging, neutralizing and washing of the specimen can be eliminated, and

3. The actual degree of resistance is perhaps more accurate, since inspissation of medium and prolonged incubation are avoided, both of which may lead to destruction of antimicrobial agents.

The fact that the method is applicable only to those sputum specimens containing tubercle bacilli demonstrable by direct smear is not so severe a limitation as it may seem upon first thought. It is precisely in those instances when patients are excreting large numbers of organisms that an early report of resistance is of most value, i.e., at the start of

therapy in a newly diagnosed case, or when the course is unfavorable. Rapid detection of resistance may become much more important in the future if primary infection with drug-resistant strains is more frequently encountered.

All photographs, with the exception of Figure 1, were taken by Mr. Julian Carlile, Rockefeller Institute for Medical Research.

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HUMAN TRIALS OF TUBERCULOSIS PROPHYLAXIS*

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PROPHYLAXIS was evidently defined in the current dictionaries before its application to tuberculosis was considered. At least "infection" and "disease" appear to be used interchangeably, a practice which can make for considerable confusion when applied to tuberculosis. This may account in part for some of the differences of opinion on the desirability of attempting prophylaxis in tuberculosis. Perhaps we are not always talking about the same thing.

We in the Tuberculosis Program of the United States Public Health Service are carrying out a series of trials. Rather than say that they are prophylactic trials, it may be wiser to describe them as an attempt to find out, as accurately and precisely as possible, what happens when apparently healthy people without clinical or x-ray evidence of tuberculosis take isoniazid daily for a year; or, more exactly, are given the opportunity of taking isoniazid for a year. I would like to describe a little of what we are doing, what we hope to learn, and why we think it is important.

Our first step in the area of prophylaxis was a half-step between therapy and prevention. It was to give isoniazid to children with asymptomatic primary tuberculosis in the hope of preventing in these children the serious complications which might accompany the primary disease. We have under observation 2750 children in 33 pediatric clinics throughout this country, in Mexico City, San Juan, Puerto Rico, and Toronto, Ontario. Half of the children were given isoniazid and half an identical placebo. Each child was to take a daily dose of 4 to 6 mg. per kilo of body weight for a year. We have assembled some preliminary data from at least three months of observation on all the children and of over a year for more than two-thirds of them. In brief, there have

* Transcript of remarks presented at the *Tenth Anniversary Meeting* of the Section on Microbiology, on *Recent Changes in the Management of Tuberculosis*, at The New York Academy of Medicine, January 15, 1958.

been 26 serious extrapulmonary complications of tuberculosis in the half of the group receiving placebo and five in the half receiving isoniazid. Of these five, two are equivocal, one because the diagnosis of the complication itself could not be verified, and the other because the child took pills for only two weeks and developed miliary tuberculosis seven weeks later.

We have, as yet, no information on what the effects will be in the year *after* medication has been taken. It is possible that the year of isoniazid will have only delayed the complications which would have appeared during the first year after the diagnosis of primary infection. As yet we have no suggestion that this will be the case. Very few complications have appeared thus far during the second year in either the isoniazid or the placebo groups.

Next, in cooperation with local health departments, we began a trial among household contacts of newly discovered cases of tuberculosis. We chose household contacts for three reasons. First was the ready acceptance of the program by health departments and the contacts themselves; health departments have acknowledged a responsibility for household contacts of new cases and they nearly all have, in varying degrees, a program that involves the supervision and observation of contacts for a year after a case is discovered in the home. The second point was a matter of economy. If we could add prophylaxis to already established programs of supervision of household contacts, the cost would be less than for establishing a program where none existed. The third reason was that contacts traditionally have been thought to have a higher-than-average risk of developing tuberculosis.

This program is now operating in 32 health departments throughout the country. Whenever a new case of tuberculosis is discovered, the members of the household are x-rayed and tuberculin tested. Any case of tuberculosis discovered at this time among members of the family does not come into the prophylactic program but is referred to the health department or a private physician for treatment. Both negative and positive tuberculin reactors are admitted to the prophylactic program.

More recently we have moved to institutional groups. So far this program has been initiated in two state mental institutional systems. Here information on whether the pills are actually taken can be obtained from the attendants who are in charge of giving medication.

The populations in both the contact and the institutional study are divided by a randomization device into two matched groups, one to get isoniazid and the other to get placebo. The bottles of pills received by the health department and institutions are labelled only with code numbers. It is a double-blind study in that neither the participants nor the persons responsible for their supervision have any notion whether a person is taking isoniazid or placebo. Therefore, in watching for the development of tuberculosis in these populations, the physicians must view with equal suspicion illnesses with symptoms of tuberculosis which develop among persons receiving isoniazid as among those receiving placebo.

All those who begin the trial will be tuberculin tested and x-rayed at the end of a year of medication. The twelve-month results plus the information on cases appearing during the year will give information on the immediate effect of isoniazid, but the more important results will come only with longer observation. Since this trial must include large numbers of people, we are not attempting to re-examine the participants after the first year. We are setting up a system whereby we shall receive a report on each case of tuberculosis and each death from tuberculosis that occurs during the ensuing years in the localities in which these persons were originally observed. We shall check that information against our roster of the participants in the trial. This alone would probably furnish inadequate information because of the great mobility of the population in this country today. In addition, therefore, we are trying to establish such rapport with persons coming into the trial that they will agree to let us know where they are every six months for the first two years and then once a year thereafter. Certain assumptions with respect to tuberculosis can be made if even limited information is obtained. If it is known that he is working, one knows that he is not in a sanitorium, that he is not dead from tuberculosis and that he is well enough to be employed.

Many participants will not remember to let us know where they are year after year. We have made arrangements so that whenever we fail to hear from someone and the health department cannot find him, a commercial credit company will, for a small fee, give us his new home address and place of employment. This may give an idea of the book-keeping task which is involved in this attempt to find out what isoniazid accomplishes when given to healthy people.

From the uninfected, those who are tuberculin negative at the time they come into the program, we hope to learn at the end of 12 months whether the use of isoniazid has prevented new infections and new disease. This information would be obtained by comparing the frequency of tuberculin conversions in the isoniazid group with that in the placebo group and by determining the amount of clinical disease appearing in both groups. Even if at the end of 12 months there should be less disease and fewer positive tuberculin tests in the isoniazid group than in the placebo group, it would be possible that the disease was only being suppressed or postponed. This possibility has led us to schedule a tuberculin test and x-ray examination again at 18 months for all those who enter the trial with a negative tuberculin test. The six-month period directly after taking the drug would seem to be the most likely time for a suppressed infection to appear.

Even if isoniazid should work almost miraculously in the uninfected, it would still be a very limited tool for the control of tuberculosis, largely because, in our view, the negative reactors today are not at great risk of being infected. The infection rate is falling rapidly. In 1950, among 100,000 Naval recruits who were tested at the time that they were being inducted, we found 9 per cent were positive to five tuberculin units. We are beginning another large survey on Naval recruits and the 3000 recruits who have been tested in the last six weeks indicate that less than 6 per cent are tuberculin positive, a drop in the prevalence of infection to only about two-thirds of what it was in 1950. We estimate that the infection rate today must be somewhere between one-tenth and two-tenths of a per cent per year. With such a limited risk, prophylactic isoniazid even if extremely effective should not be considered for general use among tuberculin negatives. It could, however, have uses in special situations in which exposure is great and unavoidable.

Among those who are already infected, the tuberculin positives at the beginning of the trial, the situation seems very different. It is estimated that in this country there are about 50 million positive reactors. This group was once widely believed to be at a lower risk of developing clinical disease than the rest of the population, but the evidence which is now accumulating indicates that positive reactors are actually at considerably higher risk. During the years since 1950, the risk of subsequently developing tuberculosis was five times as great among

the Naval recruits who came into the service already tuberculin positive as for those who came in tuberculin negative. Of the 85 thousand new cases of tuberculosis occurring in the United States each year, we estimate that about 70 per cent may come from the estimated one-third of the population which is tuberculin positive; and it seems likely that most of this disease is of endogenous origin. In Denmark, the Danish Tuberculosis Index finds very much the same picture; a high proportion of their tuberculosis also occurs in individuals who have been tuberculin positive. In the future, it would seem reasonable that an even larger share of the cases would come from the already infected, because so few of those who are tuberculin negative today will become infected. Therefore, it is of enormous interest to know what this attempt at prophylaxis will accomplish in the group who are tuberculin positive.

While pills are being taken, will we find less tuberculosis developing among those who are on isoniazid as compared with those on the placebo? Although we think this is quite likely, it seems inconceivable to us that anyone would advocate that 50 million positive reactors should take isoniazid every day for the rest of their lives. Therefore, the really important question is whether, after the medication period, less tuberculosis will develop among those positive reactors who have had a year of isoniazid. If tuberculin positive persons should be at decreased risk of tuberculosis after taking isoniazid for a year, isoniazid will be of enormous benefit to the 50 million now infected, and indirectly reduce the risk of infection among the uninfected.

There are several by-products which we hope to obtain from this study: information on the penalties, such as toxicity, which may come with widespread use of the drug; more precise information concerning the risk of tuberculosis among household contacts of an active case; and the characteristics of those positive reactors who are at greatest risk of developing tuberculosis.

A further point has to do with the problem of infection with drug-resistant strains. As Dr. Pierce mentioned, the time may come when initially drug-resistant infections become more of a problem. It is true that at present only a small percentage of new admissions to tuberculosis hospitals are found to have resistant organisms. Since perhaps two-thirds or more of the new cases may be of endogenous origin, many must have been infected before the days of chemotherapy and one would expect their organisms to be sensitive to the drugs. Among new cases that re-

sult from recent infections, resistant bacteria may be more common. Previously we have not had a record of the tuberclin status of new cases prior to their illness. But in the material from these studies, we shall be able to classify the cases appearing during the trial according to their tuberculin status at the beginning of the trial. We have arranged to send to the Trudeau Laboratory specimens for sensitivity tests on all cases. Then we can see whether there is any difference in the initial sensitivity of bacilli from persons infected before the trial and those from persons infected after the beginning of the trial.

One of the most important results of these trials is what we should learn about methodology, that is, how to improve prophylactic trials. We are meeting many new problems in these studies, and with hindsight will see that some of the ways we met these problems were inadequate or ineffective. Trials of this kind are in their infancy and this one will, in spite of all our efforts, reflect how little we yet know about how to do them. But it is a beginning, and it seems likely that there will be an increasing need for such trials as medical advances create more prophylactic possibilities.

PREVENTION OF HABITUAL SPONTANEOUS ABORTION WITH EARLY PRENATAL CARE*

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PREVENTION of habitual spontaneous abortion has interested the author for more than two decades. Perhaps a brief recital of the background of this experience will be of interest to this audience. It began in 1935, on becoming a member of the resident staff at the New York Lying-In Hospital. At first my interest was clinical, later I became concerned with the pathological aspects and more recently I have veered back to the clinical. I learned as an intern about that great American contribution to modern obstetrics, "Prenatal Care". Many textbooks of that period attributed most fetal wastage to syphilis. Stander said, "Syphilis is one of the frequent causes of fetal death in the latter months of pregnancy." Patients with a history of many abortions and stillbirths were treated with antiluetic therapy whether their Wassermann tests were positive or not. According to Hooker, who published the famous report on maternal mortality in 1933, sponsored by this Academy of Medicine, about which more will be said later, this test had not yet become a routine part of prenatal care. Nevertheless, with such empirical treatment, the pregnancy often had a successful outcome. Cross, of Dublin, Ireland, still employs this treatment at the Rotunda Hospital. I often wondered how this empirical therapy worked when the patient did not have syphilis. Was it a form of Chinese acupuncture, as described by Wong and Lien-Teh? Or was it due to other adjuncts such as prenatal care consisting of dietary measures, iron for anemia, calcium, frequent office visits, urinalysis, etc.? It did not seem logical to give the entire credit to one facet of the program, i.e., antiluetic therapy, and to ignore

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all the others. Perhaps all of the factors, taken together, produced the good results.

The current literature on habitual abortion is concerned with hormones, vitamins, or antiluetic therapy. Each regimen produces a success rate of about 80 per cent. The end results are usually discussed in terms of one agent with success attributed to it. But the outcome of the pregnancy should be weighed in terms of the entire program and not one aspect of it. One cannot build a house only of stone, one also needs wood, nails, pipes, and many other materials. It is also true that many factors play a role in the successful outcome of a normal pregnancy.

When discussing the merits of prenatal care, emphasis is usually placed on the reduction of maternal morbidity and mortality. This in turn has made conditions more favorable for the infant resulting in a lowered perinatal mortality. Seldom has the matter of prenatal care been projected into the early months of pregnancy, when spontaneous abortion, the commonest complication of pregnancy, exacts a fetal mortality rate of 100 per cent. Physicians have discouraged patients from coming to them before the fourth month because of a supposed difficulty in making the diagnosis of pregnancy.

My postgraduate training began at a time when the profession was recovering from the effects of a report entitled "Maternal Mortality in New York City" prepared by the late Ransom S. Hooker and published in 1933 by the Commonwealth Fund for the Committee on Public Health Relations of The New York Academy of Medicine. This report concluded that the maternal mortality rate was unnecessarily high, and found "first of all . . . that prenatal care was inadequate and improper". The maternal and infantile mortality rates were almost doubled when prenatal care was inadequate. The definition of prenatal care used by Hooker was that of the Childrens Bureau of the Department of Labor and it included: "First visit to the clinic or physician to be made *at* or before the fifth calendar month of gestation." It is equally distressing to read another report sponsored by the same Committee and prepared by Dr. Schuyler Kohl entitled "Perinatal Mortality in New York City". It stated "that information regarding [prenatal care] was not completely collected in the survey". Consequently, the value of prenatal data could not be appraised in an otherwise reliable study published in 1955.

Obviously, if prenatal care was good for the mother and the viable infant in the last four calendar months of her pregnancy, and good care

was desirable during labor and delivery, it could be presumed to be desirable during the first half of gestation when the fetus was not viable, but was struggling for life in utero. Under the usual regimen of prenatal care the early growth and development of the ovo-fetus had been ignored. It seemed appropriate to me to employ early prenatal care in patients with a history of habitual abortion and to begin it on a preconceptional basis. The first opportunity to employ this concept occurred in 1936 when I hospitalized such a patient for study. She was 41 years old and had experienced three spontaneous abortions at 5, 2 and 3 months respectively. The workup consisted of a blood Wassermann which was negative; a blood count, urinalysis, basal metabolism and a pelvic examination by the chief of service. Today, in addition to this workup, she would probably have a hysterogram done, an endometrial biopsy, and evaluation of the cervix, a vitamin C determination, and perhaps certain hormonal studies. This patient was told by the chief that all of the tests were negative, and that there was no physical cause for her miscarriages. She later conceived and was given routine prenatal care, including a diet of one banana and several oranges each day. She was delivered at term of an excessive sized infant weighing 4300 grams.

Habitual abortion patients were few and far between in those days and I finished my residency without seeing another one. At this time, Dr. John Pastore became interested in the prevention of blood loss in the third stage of labor. He believed that the more blood the patient had, the more she could afford to lose. Therefore, he had been conducting a Hematology Clinic to which all patients with a hemoglobin of 70 per cent or less, were sent. All patients attending the Hematology Clinic were placed on a high iron and high vitamin diet with mineral and vitamin supplements. If the hemoglobin did not respond, they were admitted to the hospital for antepartum transfusions. Gradually, patients with various blood dyscrasias, liver disease, history of postpartum hemorrhage, bleeding tendencies, bruising and bleeding gums, multiple stillbirths and erythroblastosis were also sent there. When Dr. Pastore resigned in 1940 to become an administrator, I inherited his Hematology Clinic. I persuaded the chief of service to establish, in conjunction with it, a special clinic for the study of habitual abortion patients. It became known as the Abortion Clinic and many patients actually came to this clinic in the hope of having one performed on them!

My second habitual abortion patient was seen in 1940. She had a history of five spontaneous abortions, at 2, 2, 2, 2, and 5 months' gestation, all within a relatively short period of time. She had a low blood plasma vitamin C concentration, and a low prothrombin determination in addition to being anemic from the blood loss accompanying her previous miscarriages. She was placed on a regimen of vitamins C and K, with an iron and mineral supplement. She had an uneventful pregnancy and was delivered at term. During this pregnancy she washed glassware in the laboratory in order to show her appreciation. I have since learned to recognize activity of this sort as a form of psychotherapy. Altogether, this patient has had eight treated pregnancies, six of which went to term, and two resulted in miscarriage when she wilfully omitted our program of therapy.

Incidentally, the mineral supplement proved to be very effective in preventing the expected drop in hemoglobin and cell volume values during pregnancy. This drop was attributed by Pastore and others to the physiologic hydremia of pregnancy. He believed that it could not be prevented by increasing the intake of iron, a view I was one of the first to disprove.

At this time the use of vitamin K in preventing hemorrhagic disease of the newborn, became common and I was one of the first to give it to the infant *after* delivery. Others preferred to give vitamin K to the mother. This did not seem logical to me, since at the same time, mineral oil was being prescribed for constipation. When we stopped prescribing mineral oil there was a marked increase of constipation among the patients. The medical clinic of the hospital was using a high citrus diet for constipation and we adopted it for our patients. Later on, it was used for the high vitamin C and mineral content.

These clinical experiences and many others gradually evolved into what has become a systematic program of therapy with many facets which will be outlined below. Our end results should be evaluated in terms of the entire program and not only in terms of vitamins C and K. Most of the measures have fairly sound reasoning behind them. Their value in preventing spontaneous abortion has been tested over many years. The vitamins C and K are used because of their antihemorrhagic properties, since 80 per cent of all miscarriages are complicated by bleeding.

The onset of World War II ended my activities in this Special Abor-

tion Clinic and it was ultimately closed. Following the war I returned to the Lying-In Hospital and to a new position as obstetrical and gynecological pathologist. I welcomed this opportunity to study all of the pathologic causes of abortion. I had been brought up to believe in the infallibility of the pathologist, to believe that he knew all the answers. Gradually, 2000 consecutive abortion specimens were collected and studied grossly and microscopically. Decidual hemorrhage, cord complications, the pathologic ovo-fetus, and diseases of the placenta seemed to account for most of the abortions, at least in the laboratory. Photographs and microphotographs were made while an artist worked on illustrations for a monograph. After eight years, there was enough material for the book recently published.

However, no specific pathology was found in 20 per cent of the 2000 specimens, and of these there were 18 completely normal conceptuses that were aborted by physically normal women. Why had they aborted? Conjectures on this matter led to many ramifications including a jaunt into the field of psychosomatic medicine with Dr. Beatrice Berle and Dr. Molly Harrower. We learned that many of the habitual abortion patients had psychologic conflicts about their marriage or about their husbands. Some had a domineering mother. Frequently, fear or stress preceded the miscarriage. Fear of the pregnancy, fear of another miscarriage made each pregnancy an Achilles heel. It was only natural to wonder about the vitamin therapy, was it a placebo, just as antiluetic therapy had seemed to be in the early years of my training? Were we getting results because of the "tender loving care" kind of psychotherapy in the prenatal care program, described by Myers? Berle found that confidence in the doctor played an important role, while Mann has made him a "father figure". These questions are difficult to answer, yet when one reviews the literature on habitual abortion, one is impressed by the at least 80 per cent successful outcome of the pregnancies, irrespective of the method of therapy. Perhaps the entire method is important, the tests, the teamwork, the special attention which these women seem to crave. I was reminded of the very first patient whose clinical history was related above. After an inadequate study, by today's standards, she had been assured by the chief of service that there was nothing wrong and she proved him right by having a child. The role of stress in habitual abortion is illustrated by the following case.

Case History

The patient was white, age 32, para 1, gravida 5, who had four consecutive abortions after a full term delivery. The patient had two older brothers, each of whom was born with a cleft palate. One of them died at 18 months of age following a plastic operation. The other survived a series of operations. He subsequently married and had a child with a cleft palate. This occurred after the patient's full term delivery and so unnerved her that she lost the next four pregnancies.

The sixth pregnancy was uneventful except for a new worry, the patient learned for the first time that she was Rh negative, and the husband was positive, homozygous. Large doses of vitamins A, C, and K were prescribed. The patient was seen at frequent intervals and was given positive assurances that the baby was normal. A normal female infant was delivered at term. The mother's first question was, "Is my baby normal?" For some days thereafter she shed tears of joy.

Through the years the writer has endured a lot of good natured "kidding" about the value of orange juice and vitamin C. About 1950, Dr. R. Gordon Douglas, the present Obstetrician and Gynecologist-in-Chief at the Lying-In Hospital, asked me to adopt more modern methods such as the diethylstilbestrol treatment of Smith and Smith, which was regarded as the last word on the subject. After our preliminary studies into the psychologic and psychosomatic aspects of habitual abortion had been completed, he was impressed. He revived the Special Clinic at the New York Lying-In Hospital and placed Dr. Edward Mann, a Board-qualified psychiatrist, in charge. In 1956 Mann presented a preliminary report indicating 80 per cent success with psychotherapy alone. He actually gave placebos to some patients. However, as has been the case with so many other workers in the field, he gave little credit to the prenatal, preconceptional care that all of his patients received in addition to the psychotherapy.

After 10 years as a pathologist I have become a clinician again. I have learned that many of our problems cannot be solved in the laboratory. Laboratory determinations are not dependable unless they confirm the clinical impression. We need closer observation at the bedside if we are to learn how to prevent spontaneous abortions.

Materials

The program of therapy has been tested, with minor variations, on several different groups of selected patients over a period of two decades.

There were 213 pregnancies treated in 147 habitual abortion patients, another 55 pregnancies were treated in women having a history of two spontaneous abortions; about 55 pregnancies were treated in sterility patients who have been found to have a higher than usual abortion rate. Over 100 Rh negative patients were treated in one or more pregnancies; and the series included more than 100 normal primigravidas. The program has not been employed on a large unselected patient basis, as in the out-patient department of the hospital. This would be desirable and should be done. It has not been recommended by the writer for patients with "threatened abortion" although many doctors have used it under such circumstances.

A group of 2000 abortion specimens were analyzed from the standpoint of salvage. It was my considered opinion that pathologically, 80 per cent of the specimens had been dead for some time prior to the abortion. Nevertheless, only 8 per cent of these had been regarded clinically as "missed" abortions. However, there is a salvage potential of 1 to 5 cases of abortion and perhaps in these, preconceptional, prenatal care would be effective. It might have been of value in some of the 80 per cent that died in utero.

Preventive Program

This program has been described in detail elsewhere* and will be summarized at this time:

Part I—Preconceptional Interview includes a letter from a patient describing previous pregnancies; consultation (including husband). Questionnaire. Abstracts of previous pregnancies; physical examination for detection of medical, dental, gynecologic, psychologic defects; correction, insofar as possible, of all known adverse factors, defects, deficiencies and conflicts before conception; proper spacing of pregnancies.

Part II—Dietary Measures consisting of: adequate diet, high in citrus fruits, providing 350 mg. of vitamin C daily; high calcium, iron and low salt intake. Calcium lactate and iron supplements; vitamins C, P and K supplements: ascorbic acid, 50 mg. t.i.d. or Hesper C (National Drug Company), 1 tablet containing 50 mg. of hesperidin (vitamin P) and 50 mg. of vitamin C t.i.d.; vitamin K (Synkay-

* 1. Javert, C. T. Repeated abortions, *Obstet. Gynec.* 3:420-34, 1954.
2. Javert, C. T. *Spontaneous and habitual abortion.* New York, Blakiston Div., McGraw-Hill & Co., 1957.

vite, Hoffmann-LaRoche, Inc.) 5 mg. daily. Thyroid extract only when indicated by a minus B.M.R. Concern for weight loss, rather than for excessive gain.

Part III—Psychosomatic Therapy; p.r.n. as follows: Early prenatal care; psychosomatic therapy: frequent office visits; palpation of breasts, abdominal palpation, vaginal speculum examination p.r.n. Auscultation of fetal heart (also by patient). "Tender loving care" (t.l.c.); unlimited phone calls, group therapy, scrapbook of cases, constant encouragement, mutual faith and confidence; relaxation of the patient and her uterus. Peace, tranquility; meprobamate, 400 mg. b.i.d. Sedation: wine, beer, alcohol, phenobarbital, p.r.n. Smith-Hodge pessary for retroversion; hospitalization, p.r.n.

Part IV—Some Don'ts, including no hot tub baths, heating pads, hot water bottles or ice caps; no complete bedrest (except for threatened abortion); no sex hormones (diethylstilbestrol or progesterone); no vitamin E; no mineral oil; no abdominal girdles; no empirical antiluetic therapy; no coitus during entire pregnancy; no smoking.

Part V—Surgical Therapy

SURGICAL THERAPY EMPLOYED IN 187 HABITUAL ABORTION PATIENTS

Abdominal	*No. of Cases*
Uterine Suspension	2*
Myomectomy	5
Unification Operation	2
Presacral Neurectomy	0
Vaginal	
Incompetent Cervix	6
Emmet Repair of Laceration	3
Schirodkar Operation	1
Total	19 or 10 per cent

* Pessary was used in 30 other patients.

Results

The first results obtained with this prenatal program of preventive therapy were published in 1949 by Javert, Finn and Stander on 48 habitual abortion cases with 87 per cent success. Further experience was reported by the author in 1954 when a 95 per cent salvage was

TABLE I.—HABITUAL ABORTION RESULTS BEFORE AND AFTER THERAPY

			Type of Delivery									
	No. of Cases	No. of Pregnancies	Spont. Abortion		I or T* Abortion	Immature	Premature	Full Term		Total Viability	
			No.	%	No.	No.	No.	No.	%	No.	%
Before	187	712	654	91.8	9	1	10	38	5.3	49	6.9
After	147	213	41	19.2	2	7	12	151	70.9	170	80.0

* Induced or Therapeutic.

obtained in 43 additional cases. The series has now been extended to a total of 187 cases of habitual abortion. These have been interviewed (usually with their husbands), 147 of them have completed 213 pregnancies, and ten additional cases are undelivered. They had 170 viable infants, a success rate of 80 per cent, while 19 per cent have continued to miscarry, about double the usual incidence. Previously, this group had experienced 712 pregnancies of which 92 per cent had ended in spontaneous abortion. These data are shown in Table I.

The gradual decrease in the percentage of success in the author's habitual abortion series, from 87 in 1949, 95 in 1954, to 80.0 per cent in 1957 can be attributed, in part, to the fact that the earlier reports included too small a number of patients. However, six of these patients are "perpetual aborters" who had 27 abortions before the author's therapy and 12 thereafter. These patients account for nearly a third of the 41 failures shown in Table I. One of these has been referred to Dr. John Rock in Boston. Another has had the Lash procedure. It should be mentioned that 40 women, or 20 per cent of the total number, have either refused to attempt more pregnancies, or they have developed a secondary infertility. Some were at or near the menopause. Several have been aided in adopting children. For some, habitual abortion has become an Achilles heel. On the other hand, 37 other cases have been delivered two or more times. Two others have had induced abortions!

The group of 55 women with a history of two consecutive abortions have also had a success rate in the neighborhood of 80 per cent.

The total abortion rate in more than 500 treated patients was not established since this group was selected and heavily weighted with habitual abortion, sterility and Rh negative patients. Sterility patients

have a miscarriage rate of 25 per cent, according to MacLeod, while the habitual aborters in this study had lost 92 per cent of their previous pregnancies.

Conclusions

A system of preconceptional prenatal care, including vitamin and mineral supplements, psychotherapy and a good doctor-patient relationship has produced successful outcome in 80 per cent of 213 pregnancies in 147 cases of habitual abortion.

Preconceptional care is an important adjunct to prenatal care in the prevention of fetal mortality in non-viable infants of habitual abortion patients.

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JOHN WAKEFIELD FRANCIS, 1789-1861
THE ACADEMY'S SECOND PRESIDENT

"Who does not know the venerable Doctor?, the Mentor of the profession, the kindly assistant of the young aspirant. . . . The Doctor is the centre of New York, and his presence is necessary to every public meeting. The antiquarians, the printers, the politicians, the literati, the artists, the 'Knickerbocker', gentlewomen, the men in rule, his own profession, all look to him as an essential to their counsels, their circles, their well-being." These seemingly over-enthusiastic, but really little exaggerated sentiments were written in 1850 by the New York correspondent of a New Orleans journal. The venerable doctor was John

Wakefield Francis, genial, witty, compassionate, whose high place in the medical profession did not deter his ready participation in other activities and friendly fellowship with leading figures of his day.

In 1789 he was born in New York, the city he came to love so well. The death of his father, a German immigrant, forced him to become briefly a printer's apprentice to help in the support of his family. He was fortunately able to return to his studies, receiving his A.B. degree from Columbia in 1809 and his M.D. in 1811. A student of David Hosack, he soon became his partner. He served as professor of medicine and materia medica when the College of Physicians and Surgeons merged with the Medical Department of Columbia in 1814, but interrupted his career for a prolonged visit to Europe in 1816-17. Here he studied under or met such distinguished physicians as Sir Astley Cooper, Abernethy, Cuvier, Gall, and Bell. Again in New York, he resumed his teaching, now adding the subject of forensic medicine and obstetrics. In 1826 he shared the difficulties of the ill-fated Rutgers Medical College, and by 1830 had retired from teaching to devote himself to the extensive practice and busy professional life which led Marshall Hall to call him the "most representative physician of his generation."

On November 10, 1847, at the Broadway Tabernacle, Dr. Francis delivered the Academy's first Anniversary Discourse. The oration fills 112 printed pages and gives an interesting picture of the history of medicine in New York. It lasted two hours, but was enlivened by appropriate selections performed by Dodsworth's cornet band. In his recapitulation Dr. Francis remarked that in 1691 the population of the city "was 4202, (a little over the number of this audience)." Four thousand is a large figure for an Academy audience, but even so, according to an account of the meeting, "hundreds must have gone away disappointed, being unable to obtain places." A few months later, Dr. Francis spoke again, when on February 2, 1848, he gave his inaugural address as second president of the Academy. This briefer message reminds his listeners that the "Academy seems to be already extensively known: its brief history is widely circulated in many periodicals; favorable views of its objects have been promulgated; the people awaked to its action; our City Council have, on several occasions, appealed to its decisions." These sentiments did not deter his refusal in 1855 when for a second time he was elected president. He declined because of the recent death of his son, but was prevailed upon to accept,

and he did preside at several meetings. In 1857 he took part in the puerperal fever controversy which was heatedly carried on at more than one meeting. He believed that the disease was contagious, citing cases where it was apparently transmitted by the hands and clothing of physicians. This must have been of particular interest to him, as he had a large obstetric practice.

Dr. Francis died on February 8, 1861. At a special meeting held on the following day it was resolved that the Fellows of the Academy should "attend his remains to their last resting-place, wearing the usual badge of mourning." His career had been long and successful, but histories of medicine will not record his achievements. He is remembered today for his excellent history of New York, *Old New York*, 1858. He served as one of the editors of the *American Medical and Philosophical Register*, 1810-1814, and as editor of Thomas Denman, *An Introduction to the Practice of Midwifery*, New York, 1821. He wrote a few papers and addresses. Garrison mentions him only briefly in his history; Castiglioni, Major, Mettler, not at all. But in the first volume of the new series of the Academy *Bulletin*, 1925, pp. 35-40, Garrison, at the suggestion of Osler, wrote a sketch of this man whose "position in the more general history of medicine could be stated in three lines." As a personality, however, he "was very remarkable, and filled the public eye for half a century." His familiar figure was known well to the literary and artistic luminaries of the city. Equally well known was he to the poor in the slums. In looks he bore a marked resemblance to Benjamin Franklin. In the Malloch Rare Book Room of the Academy Library stands a small figure of a pleasant looking man with hat in hand. The casual observer would surely identify it as Franklin, but it is James Trudeau's statuette of Francis, the leading physician of New York in his day whose funeral was attended by "rich and poor, savant and mechanic, physician and author, merchant and printer, the actor and the preacher, the statesman and the apprentice."

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NEW YORK HEART ASSOCIATION

ABSTRACTS OF PAPERS

Presented at the Scientific Session held at The New York Academy of Medicine, April 22, 1958

Plasmin Lysis of Experimental Coronary Thrombi

PAUL RUEGSEGGER, IRWIN NYDICK, ALVIN FREIMAN, NILS BANG, STEVEN KANOR, EUGENE E. CLIFFTON AND JOHN S. LADUE

The Sloan-Kettering Division of the Cornell University Medical College, New York, N. Y.

The effects of the fibrinolytic enzyme plasmin upon experimental coronary thrombi and the myocardium were studied in open chest dogs by direct coronary arteriography and histologic examination.

The minute anatomy of the coronary vessels was delineated by means of serial arteriograms by the injection of Hypaque through a #190 polyethylene catheter inserted into a small proximal side branch of the major vessel under study during control periods and after coronary blockade. This permitted repeated roentgenographic demonstration of the coronary tree with its occluded segment throughout the experiment.

The coronary thrombi were produced by inducing a "hypercoagulable state" in a distal isolated segment of a major artery by modification of the method previously described by Sanford Wessler. Intracoronary clotting occurred within two minutes following the injection of a mixture of blood and serum from the operated animals. In control experiments, the clots were relatively unchanged for 10 to 12 hours. Clot retraction of approximately 30 per cent was noted in one animal.

Complete or partial lysis of coronary thrombi resulting in restoration of blood supply to the ischemic myocardium was achieved in one to six hours in 100 per cent of plasmin treated animals. These animals received 4,000 plasmin units/kg/hour by systemic administration. The blood of all plasmin treated animals showed strong fibrinolytic activity.

Gross and microscopic examination of animals autopsied within 12 hours after occlusion revealed striking differences between the two groups. The untreated hearts in addition to the primary thrombus showed marked capillary engorgement with frequent thrombi and interstitial edema of the infarcted and marginal ischemic zones, whereas the plasmin treated hearts showed edema only. The late effects of these observed changes upon muscle cell damage are under study. There was no pathological evidence that plasmin caused hemorrhage or any other injury to the heart structures.

The Pathogenesis of Cor Pulmonale in Kyphoscoliosis (Abstract)

EDWARD H. BERGOFSKY, GERARD M. TURINO AND ALFRED P. FISHMAN

Department of Medicine, Presbyterian Hospital, New York, N. Y.

Hypertrophy and failure of the right heart frequently evolves in patients with kyphoscoliosis. The relationship between the chest deformity and cardio-respiratory performance was investigated in 27 patients by the following methods: 1) cardiac catheterization for measurement of pulmonary blood flow and vascular pressures, 2) spirometry for the measurement of lung volumes, 3) analysis of expired gas and arterial blood in gas exchange and 4) passive, artificial ventilation in a tank respirator for the determination of the mechanical work of moving the thorax.

The results of such studies indicate that pulmonary blood flow is being funnelled into a diminutive vascular bed. The volume of the vascular bed is apparently reduced in proportion to the small size of the lung. On this background, other mechanisms which increase cardiac output or elicit pulmonary hypertension, such as the hypoxemia, polycythemia and hypervolemia of ventilatory insufficiency, may contribute to the evolution of cor pulmonale. Back pressure from the left heart, as measured by the "wedge" pressure, does not contribute to the pulmonary hypertension.

The deformed chest wall results in severe ventilatory insufficiency, which is manifested by alveolar hypoventilation. This abnormality stems from a compensatory breathing pattern employing high frequencies and low tidal volumes rather than from intrinsic lung disease. Although such a pattern reduces the excessive work and energy cost of breathing, it is detrimental in terms of the hypoxemia and hypercapnia which it induces. Hypoxemia and its sequelae strain the right heart, while chronic hypercapnia diminishes the sensitivity of the respiratory center to the carbon dioxide stimulus.

The therapeutic implications are: 1) sedatives, which depress the respiratory center, or pure oxygen which decreases the ventilatory stimuli, may induce carbon dioxide narcosis and death, 2) alveolar hypoventilation may be relieved by mechanical respirators despite the skeletal deformity and 3) pulmonary hypertension and heart failure may be minimized by relieving hypoxemia and polycythemia.

Detection of Circulatory Shunts by Use of a Radioactive Gas (Abstract)

PAUL KEATING, ROBERT CASE, H. L. SACHS

St. Luke's Hospital, New York, N. Y.

A new method of detection of left to right circulatory shunts by the use of a radioactive gas is described which appears to obviate the known inaccuracies of the oxygen "step-up" method. Using the gas ethyl iodide containing radioactive I-131 a total of 14 studies were made on dogs with acute or chronically prepared atrial defects and normals. Catheters were placed in the pulmonary artery, femoral artery and vena cava. Simultaneous blood samples were taken every five seconds during inhalation of the radioactive gas in a closed system. Exhaust was collected in polyvinyl bags or a series

of bottles containing ethyl alcohol in which the gas is absorbed. One cc. samples were measured for radioactive content with a scintillation detector. Results in normal dogs show the expected initial high arterial values and low pulmonary artery and vena cava values. Later values from three sampling sites tended to approach one another as recirculation occurred. Results in shunt dogs indicated initially high pulmonary arterial levels which were sometimes in excess of femoral arterial values. Femoral artery values were compared at an optimal time before recirculation became a factor. In normals pulmonary artery values averaged 1.6 per cent of femoral artery values with a range of 0-4.9 per cent, whereas shunt dogs averaged 43.9 per cent with a range of 20-60.6 per cent. These values gave a clear indication of the presence or absence of an atrial defect and presumably the method would apply to other types of left to right shunts. We are now using radioactive ethyl iodide for the detection of left to right shunts in humans. Its lack of toxicity and the rapidity and accuracy of analysis are additional desirable factors. The size of the shunt may be calculated by a simple formula using only pulmonary artery and femoral artery concentrations of radioactivity.

*The Effects of Safflower Oil Emulsion on the Serum Cholesterol Levels in Young Adult Males When Administered as a Supplement to a Normal Diet (Abstract)**

RICHARD PERKINS, IRVING S. WRIGHT AND BARBARA GATJE, B.S.

Vascular Section of the Department of Medicine, New York Hospital-Cornell Medical Center

Large numbers of patients are now taking various unsaturated vegetable oil emulsions as supplements to their regular diets, in hopes of reducing their serum cholesterols. Although the careful *substitution* of unsaturated fat for saturated animal fat, in experimental diets, has proven to be effective, little experimental evidence is available that dietary supplements of unsaturated vegetable oils will work in the same way.

Safflower oil, with an iodine number of 143, is the most unsaturated vegetable oil available, and it was chosen by us to be used, in a 65 per cent emulsion, as a supplement to the usual diet of 24 normal young adult males. A sucrose placebo was also prepared, and was incorporated into the experiment.

The regular diets of the subjects were determined before the experiment began. Serum cholesterols were determined weekly. After a preliminary control period of 5 weeks, the subjects were divided into two groups. Twelve took 75cc. of safflower oil emulsion daily (about 48 grams of oil, the usually prescribed dose); twelve took the same amount of placebo. After seven weeks of therapy, the two groups were reversed. The total length of the experiment was 18 weeks.

The results showed a considerable variability in individuals' total serum cholesterol from week to week, indicating the importance of a fairly long control period, with several cholesterol determinations, in such studies. During the administration of both safflower oil and placebo, the average cholesterol of the group slowly increased from week to week. The safflower oil emulsion as a supplement to the usual diet had no demonstrable depressant effect upon the total serum cholesterol levels of these 24 young adult males.

* Abstract of an article published in J.A.M.A. *166*:2132, 1958, printed in abstract form by permission.

The Prognosis of Rheumatic Carditis (Abstract)

ALVAN R. FEINSTEIN AND RODOLFO DI MASSA

New York University College of Medicine, Irvington House, Irvington-on-Hudson, N. Y.

The present study was done to learn which clinical features are most significant in predicting the outcome of an attack of rheumatic carditis. The patients were 315 children and adolescents observed at monthly intervals at the Irvington House Prophylaxis Study Clinic following an initial attack of unequivocal rheumatic fever, without a recurrence. The follow-up period has averaged 4.8 years (range 2-9 years) and 78 per cent of the patients have been followed for 4 years or more.

The features of the acute attack were carefully analyzed for evidence of "carditis", and the patients were classified according to the *murmurs present* during the attack. The designation "valvulitis" was given to 96 patients, without previous heart disease, in whom a definite diastolic murmur appeared or disappeared during the acute attack. Of these, 63 (66 per cent) now have rheumatic heart disease. The designation "probable valvulitis" was given to 52 patients who developed: 1) a new loud apical systolic murmur radiating to the axilla but no diastolic murmur or, 2) a questionable diastolic murmur. Of 52 patients in this group, 15 (29 per cent) now have heart disease.

The designation "no valvulitis" was given to 167 patients who did not meet the above criteria for valvular involvement, although most of these patients had other systolic murmurs and many had prolonged P-R intervals, gallop rhythm, "changes" in cardiac size and other features of rheumatic "carditis". Of these patients, none has rheumatic heart disease today.

Many more years of further observation will be needed to confirm these results. However, the existing data strongly indicate that the auscultatory phenomena present during the acute attack of rheumatic fever are of primary importance in predicting the cardiac prognosis. Patients without "valvulitis" or "probable valvulitis" may be reasonably assured subsequent heart disease will not develop in the absence of rheumatic recurrences. The results also indicate renewed importance for the stethoscope as a diagnostic tool.

Wedge Pulmonary Arteriography: Application in Congenital and Acquired Heart Disease (Abstract)*

A. L. LOOMIS BELL, JR., SEIICHI SHIMOMURA AND W. JAMES GUTHRIE

Cardiopulmonary Labortory, St. Luke's Hospital, New York, N. Y.

A clinical method for visualizing radiographically the smaller pulmonary arteries and arterioles has been devised. At right heart catheterization, the cardiac catheter is wedged into a pulmonary artery and an x-ray exposure made after injection of 3 cc. of 70 per cent Urokon, using non-screen film at 90-110 kv, 300mA, and 1/30 second exposure.

This technique has been applied in the study of 25 cases with cardiac defects showing hemodynamic patterns of: 1) normal pulmonary artery pressures and increased pulmonary flow, 2) high pulmonary artery pressure and increased pulmonary blood flow, 3) high pulmonary artery pressure and decreased pulmonary flow, and 4) increased pulmonary artery pressure due to increased left atrial pressure. The arteriograms of these patients have been correlated

* Aided by a grant from the New York Heart Association.

with the hemodynamic studies and with lung specimens obtained at surgery or autopsy.

The normal pulmonary arteriogram shows abundant arborization of small arteries of 200-2000 micra, with filling of smaller arterioles and capillaries producing distinctive background markings. The abnormal pulmonary arteriogram of patients with pulmonary hypertension and balanced or right-to-left shunt, shows sparsity of arborization of these small pulmonary arteries, abrupt termination of 800-1200 micra arteries, or abrupt narrowing of these arteries into fine, tortuous channels without normal capillary filling. The cases with normal pulmonary artery pressure and either low or high pulmonary flow may show no changes as compared with the normal. The abnormal arteriogram shows an excellent correlation with the histological picture of intimal proliferation and occlusive changes seen in pulmonary arteries of 300-1000 micra diameter in those patients with high pulmonary artery pressure and decreased pulmonary blood flow. The arteriograms of patients with pulmonary artery hypertension due to left heart obstruction may show decreased arborization of an intermediate degree.

These preliminary studies indicate that wedge pulmonary arteriography may have real value in demonstrating radiographically occlusive intimal changes in certain cases of secondary pulmonary hypertension. The method should prove useful in selecting patients with congenital cardiac defects in whom correction of the defect is feasible.

Microelectrode Studies of the Mechanism of A-V Nodal Delay (Abstract)*

BRIAN F. HOFFMAN AND PAUL F. CRANEFIELD

State University of New York, Downstate Medical Center, Brooklyn, N. Y.

Multiple intracellular microelectrodes have been employed to record transmembrane action potentials simultaneously from single fibers of the atrium, atrio-ventricular node and His bundle of rabbit and dog hearts. The tissue preparation, consisting of the S-A node, right atrium, interatrial septum, and upper interventricular septum, was maintained in a modified Tyrode solution at 35°C. Electrical activity of single fibers of the A-V node was recorded during normal A-V transmission and during varying degrees of A-V dissociation induced by high atrial rates, acetylcholine and hypoxia.

Action potentials recorded from fibers of the A-V node are distinctive in shape and differ from those of either atrial muscle or His bundle. At the atrial border of the node the transmembrane action potential is characterized by a low rate of depolarization and a reduced overshoot. Resting potential is low and the rising phase of the action potential is frequently slurred or notched. Action potential duration is not greater than in atrium and slow diastolic depolarization is present. The slow spread of activity is mainly localized to this area which is 1 mm. or less in length. Apparent conduction velocity may be as low as 0.05 to 0.02 m/sec. Between this site and the upper His bundle there is a gradual transition in action potential shape to that typical of Purkinje fibers.

During partial or complete A-V dissociation caused by acetylcholine, failure of transmission is localized to the atrial end of the A-V node. Single fibers in this area show depolarizations of graded amplitude resembling electrotonic spread or local response resulting from atrial or His bundle activity. Ventricular escape results from pacemaker activity in fibers of the His bundle. During high atrial rates and hypoxia block is similarly associated with graded activity. Records of nodal activity are compatible with the interpretation that transmission across a narrow area results from decremental conduction.

* This work was supported in part by a grant from the New York Heart Association.

MONTHLY PANEL MEETINGS FOR THE GENERAL PHYSICIAN

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(Please note: For the coming academic year these meetings will be held on the SECOND FRIDAY of the month, November 1958 through April 1959 at **4:00 p.m.**, *in Room 20 at the Academy.)*

FRIDAY November 14 1958	THE USE AND ABUSE OF TRANQUILIZERS *Moderator:* JOHN M. COTTON
FRIDAY December 12 1958	CURRENT THERAPY OF PULMONARY TUBERCULOSIS *Moderator:* JOHN H. MCCLEMENT
FRIDAY January 9 1959	ADVANCES IN THE MANAGEMENT OF LYMPHOMAS *Moderator:* CLAUDE E. FORKNER
FRIDAY February 13 1959	USE OF STEROIDS IN RHEUMATIC DISEASES *Moderator:* CHARLES RAGAN
FRIDAY March 13 1959	THE USE OF CHLOROTHIAZIDE AND OTHER DIURETIC AGENTS *Moderator:* JOHN H. LARAGH
FRIDAY April 10 1959	PROPHYLACTIC USE OF ANTIBIOTICS *Moderator:* PERRIN H. LONG

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BECKER, M. C., Simon, F. and Bernstein, A.: J. Newark Beth Israel Hosp. **9**:58 (January) 1958.

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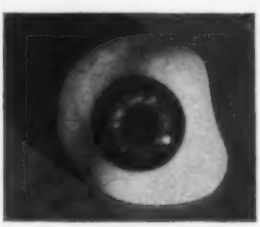

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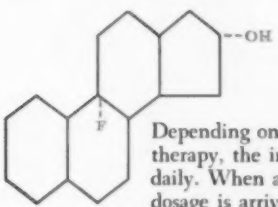

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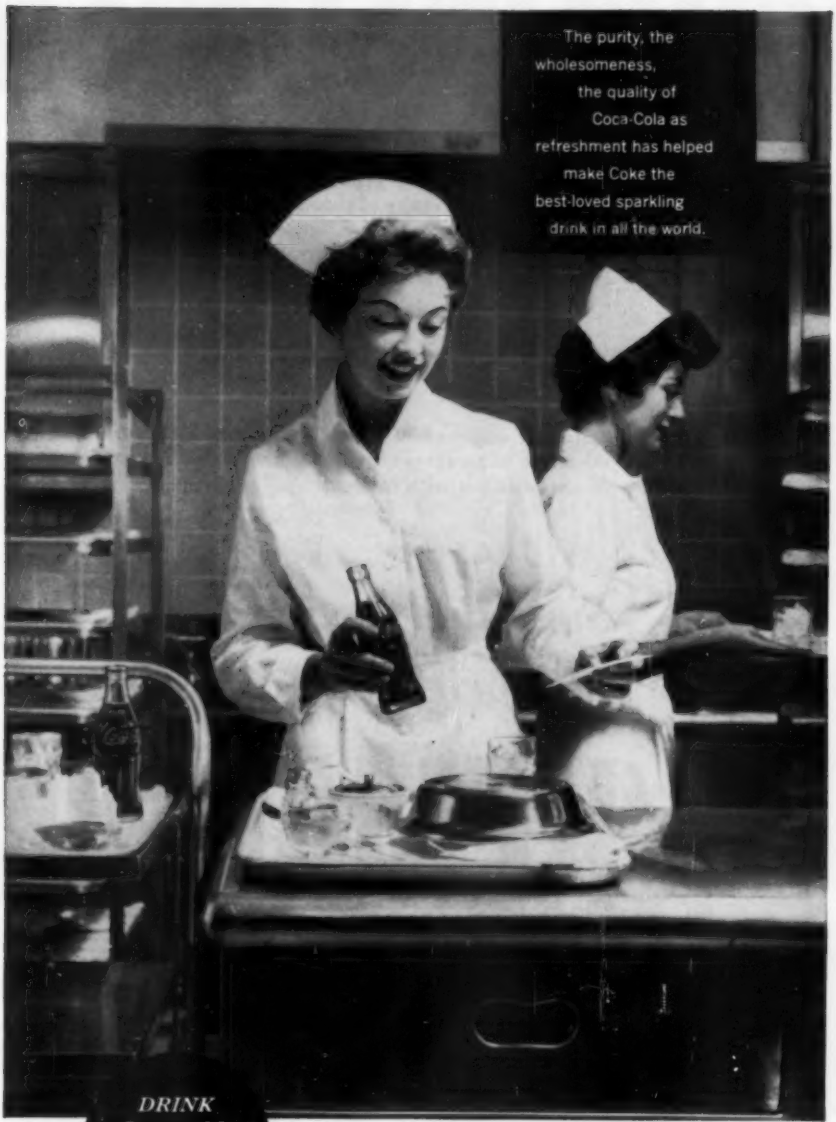
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Lerner, P. F.: Kemadrin, a New Drug for Treatment of Parkinsonian Disease, J. Nerv. & Ment. Dis. 123:79 (Jan.) 1956.

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1. Kety, S. S.: A medical regimen for benign rectal disorders, *GP* 10:75, Nov., 1954.

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Wilkins, R. W.: New England J. Med. **257**:1026, Nov. 21, 1957.

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Freis, E. D., Wanko, A., Wilson, I. H. and Parrish, A. E.: J.A.M.A. **166**:137, Jan. 11, 1958.

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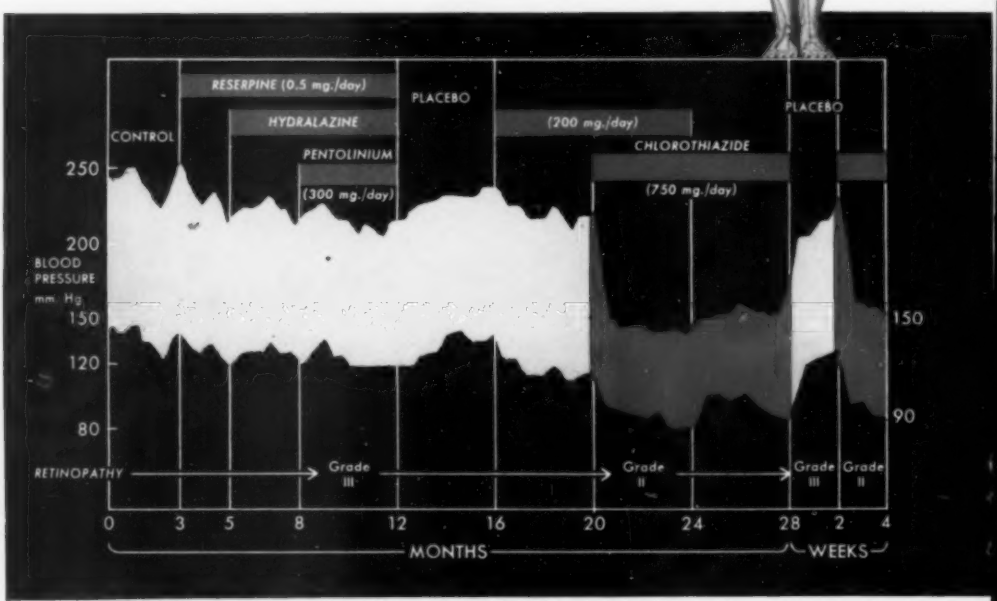

In "Chlorothiazide: A New Type of Drug for the Treatment of Arterial Hypertension," Hollander, W. and Wilkins, R. W.: Boston Med. Quart. **8**: 1, September, 1957.

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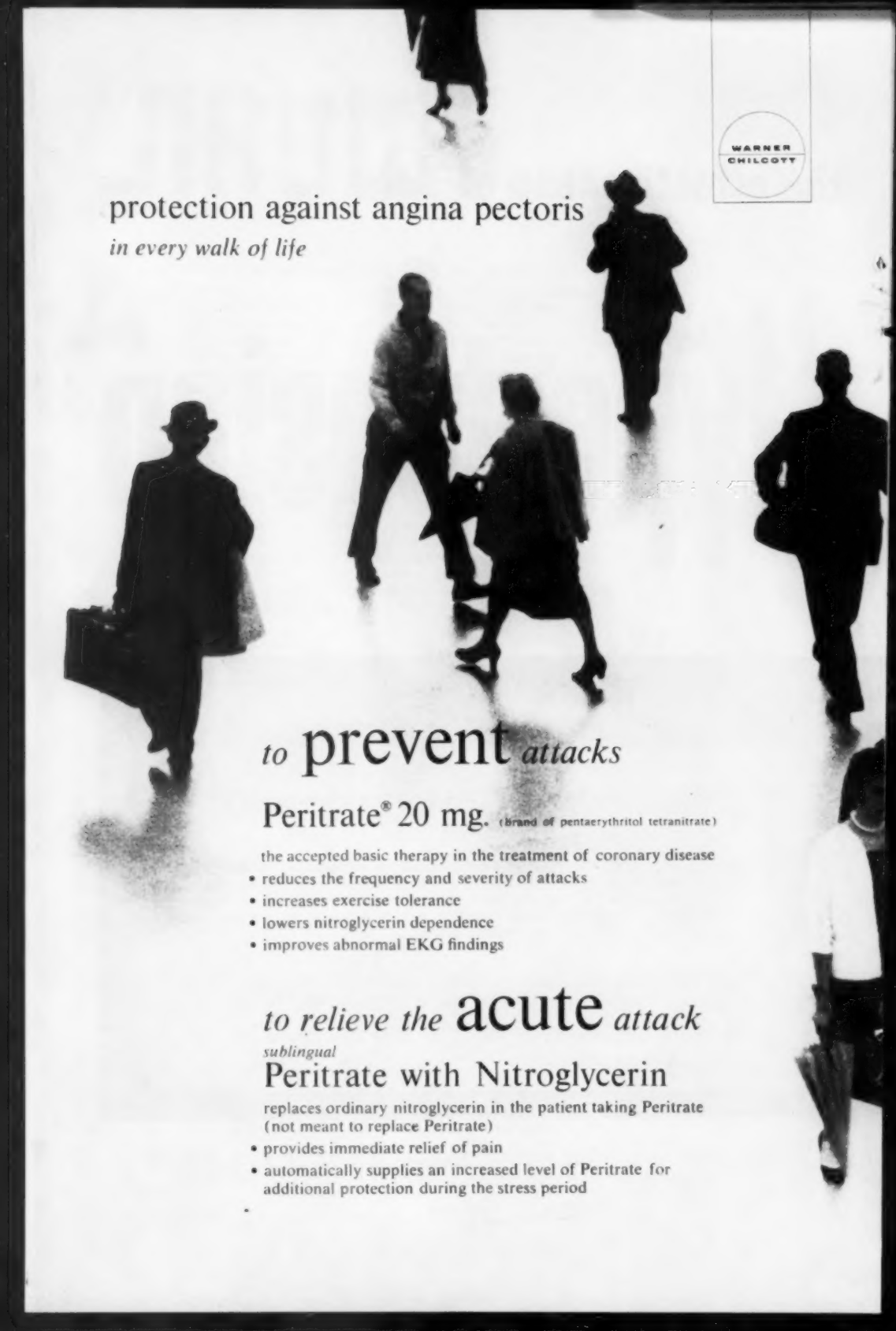
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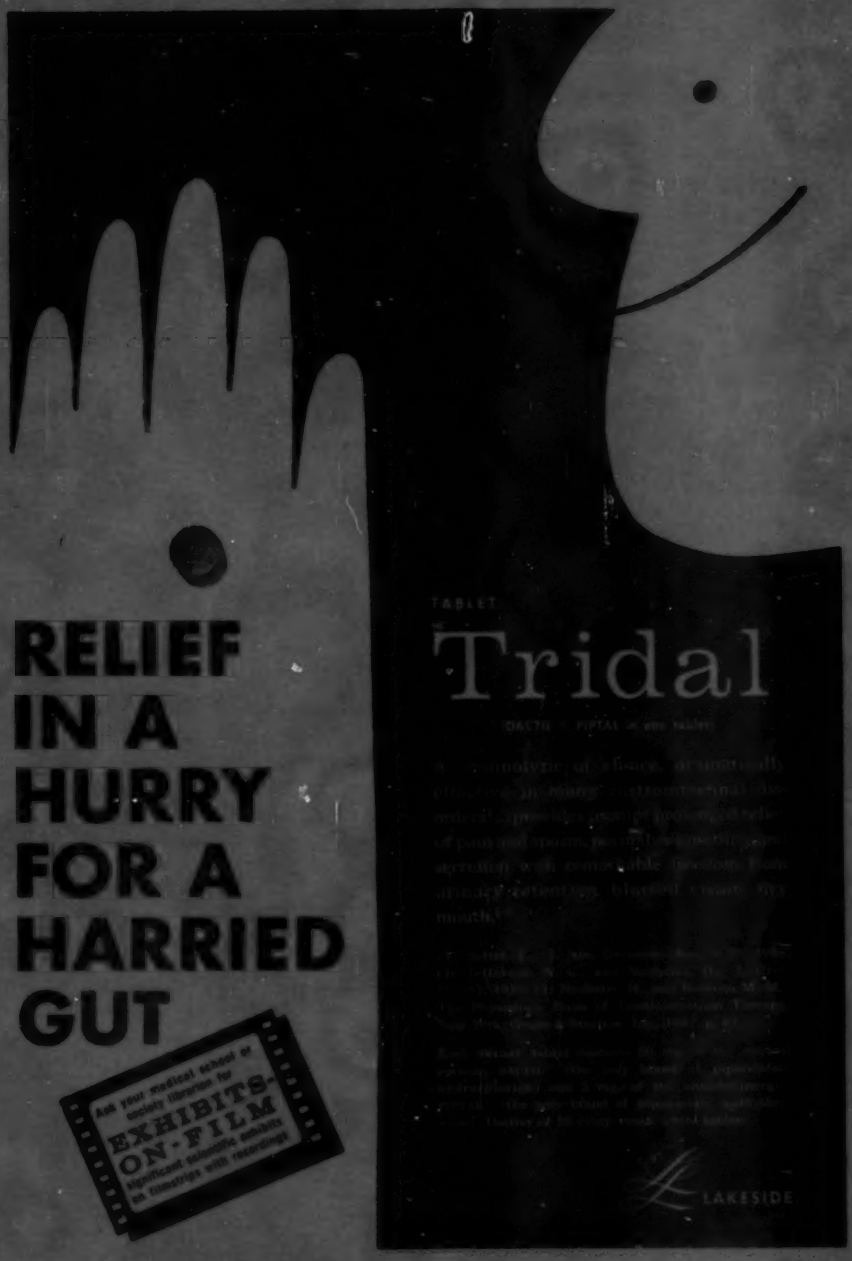

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